EXTENDING SHELF LIFE OF BEEF CUTS UTILIZING LOW LEVEL CARBON MONOXIDE IN MODIFIED ATMOSPHERE PACKAGING SYSTEMS

By

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FORMAT OF THESIS

This Thesis is presented in the Journal of Animal Science style format, as outlined by the Oklahoma State University graduate college style manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER I

INTRODUCTION

Carbon monoxide (CO) is a colorless, odorless, and tasteless gas. CO is also considered a toxic gas; therefore, its use for food packaging is not allowed in most countries. However, the Norwegian meat industry has been using a gas mixture containing 60-70% CO_2, 30-40% N_2, and 0.3-0.4% CO, for the past 20 years; indeed, it is estimated that 50-60% of the retail meat and 85% of ground beef is packaged under this modified atmosphere regime (Norwegian Food Control Authority, 2001).

The Food and Drug Administration approved carbon monoxide as a substance that is Generally Recognized as Safe (GRAS) in 2001. It was approved at the level of 0.4% in modified atmosphere packaging (MAP). The other required components of the MAP system are carbon dioxide at a set level of 30% and nitrogen at a set level of 69.6%.

Meat appearance determines how consumers perceive quality and significantly influences purchasing decisions. In the case of beef, the two important visual clues that determine perceived quality are color and packaging (Issanchou, 1996), and consumers prefer to purchase bright red beef rather than purple or brown beef (Carpenter, et al. 2001).

Many gas combinations cause substantial increases in the shelf life of meat; however, according to Sorheim (1997) in today's meat industry discoloration of meat products can be prevented by the inclusion of a low level of CO in the modified gas mixture. The main function of low levels of CO in MAP is to give meat a stable, cherry red color (Lentz, 1979). Since CO's reaction with meat does not require oxygen; this could be a way of maintaining the desired color of prepackaged fresh beef in the absence or near absence of oxygen, and thus retarding oxidative rancidity, which adversely affect flavor.

Research has shown that packaging at a level of 0.5% CO resulted in a stable, red lean color for >30 days, whereas discoloration occurred after 5 days of storage in control samples packaged in air (Clark, et al., 1976). In addition, the shelf life with respect to odor was prolonged with increased CO concentration over that of meat stored under nitrogen, which indicated that CO directly inhibited the growth of inoculated psychotropic bacteria, which was added to the samples evaluated. In beef loin steaks in 0.4% CO/ 60% CO_2/ 40% N_2 odor stability lasted four days longer than steaks stored in 70% O_2/ 30% CO_2 (Sorheim et al., 1997).

Concerns about the use of CO in modified atmosphere packaging focus on the phenomenon of "persistent pinking" in cooked product and potential toxicity. Previous research by Marksberry (1990), Van Laack and others (1996b), and Berry and Bigner-George (1999) observed that beef patties treated with varying levels of CO remained pink when cooked to 74ºC or higher. This undesired red or pink color, commonly referred to as persistent pinking, is present immediately after cooking. Persistent pinking causes quality issues due to the consumer overcooking their product treated with CO, and thus reducing the tenderness and juiciness of the product.

High level of CO can create toxicity problems for consumers when they eat meat packaged with CO. The Pactive Corporation (Lake Forest, IL), the company that proposed the usage of CO in MAP packaging in the U.S., estimates the "worst case scenario" intake to be 1.88 mg CO per meal. This assumes that 100 percent of the CO present in MAP is absorbed into the meat and no reduction occurs during cooking (USDA, 2001). Watts and others (1978) reported that beef that had been treated with CO at a level of 1% for three days yielded approximately 0.1 mg CO per kilogram of meat upon cooking.

Based on this information, the objectives of this study was to assess the impact of low-oxygen packaging systems containing CO on shelf-life of beef cuts; determine if sensory ratings are altered by a case ready system containing CO; compare effectiveness of CO versus current packaging systems on microbiological profile; and examine if CO retards the formation of metmyoglobin ("browning") and oxidative rancidity properties in case ready packaged beef cuts.

CHAPTER II

LITERATURE REVIEW

Importance of Color in Today's Meat Industry

Visual appearance is one of the major criteria used by consumers to assess the quality and palatability of a meat cut (Howe et al., 1982). Appearance determines how consumers perceive quality and significantly influences purchasing decisions. In the case of beef, two important visual clues that determining perceived quality are color and packaging (Issanchou, 1996). According to Carpenter et al. (2001), there is a close link between lean color preference and the decision to purchase, and consumers prefer to purchase bright red beef rather than purple or brown beef. Furthermore, consumers use color as an indicator of beef freshness, and will make a no-purchase decision when brown metmyoglobin reaches 30 to 40% of total pigments on the surface of fresh beef (Gee et al., 1981). Color and discoloration of fresh meat does not necessarily reflect nutritional, flavor, or functional values (Zhu & Brewer, 1998).

Meat color is largely dependant on the chemical state of myoglobin (Mb). In the reduced form, deoxymyoglobin is purplish; in the oxygenated form, oxymyoglobin (OMb) is bright red; and in the oxidized form, metmyoglobin (MetMb) is brown after storage (Govindarajan, 1973). During refrigerated storage, three forms of Mb can interconvert through oxygenation, oxidation and reduction reaction. In general, low pH values favors oxidation of Mb (Brown and Mebine, 1969; Gidding, 1977) and decreased enzymatic reduction of metmyoglobin (Ledward, 1992). Therefore, the accumulation of metmyoglobin is faster in meat with a low pH than in meat with a high pH and meat tends to discolor more readily in the low pH condition (Owen and Lawrie, 1975; Ledward, 1986).

In living animal, Mb must be in the reduced form to remain active in the transportation and storage of oxygen. Many oxidants have the capability of oxidizing Mb, thus making it physiologically inactive. However, in living systems, practically no MetMb is present because of an inherent MetMb-reducing system (Gidding, 1977). Although chemical properties change when muscle is converted to meat, Mb can still be reduced. However, the relationship between metmyoglobin reducing ability (MRA) and meat color stability is unclear. Logically, the more MRA a muscle retains, the better its color stability will be (Sammel et al., 2002).

Exposure to light also influences meat discoloration during storage (Kropf, 1980). Meat surfaces accumulate more MetMb in the light than in the dark (Lawrie, 1991; Zhu, 1997). Instrumental measurement techniques are commonly used to evaluate surface color of fresh meat and the efficacy of individual measures has been reported (Zhu, 1997; Zhu and Brewer, 1998).

Bright red beef out-sells discolored beef (20% or more surface MetMb) by a ratio of 2:1 (Hood and Riordan, 1973). According to Andersen and others (1989), rates of product discoloration depended on the intensity of light and wavelength distribution used for retail display, in combination with the light permeability of the packaging material. Lentz (1979) found that frozen retail beef cuts exposed to light (0 to 2,000 lux) had a color shelf life of 1 day to more than 90 days, depending on light intensity and temperature. The color fading in beef steaks displayed in chill cabinets has been found to be caused by the combined action of tension, surface microbial growth, temperature and type of lighting (Renerre, 1990).

Several studies have examined the effects of light on discoloration of fresh meat, although they reported conflicting results. Zachariah and Satterlee (1973) and Solberg and Frank (1971) demonstrated that visible light (500 to 600 nm), at 540 to 2,150 lux, caused a small but significant increased in the accumulation of MetMb at the surface of meat. Lentz (1971) reported that lean color of frozen beef remained attractive for 3 months when stored in the dark, but only 3 days when stored under illumination of 1,600 to 2,100 lux. Satterlee and Hansmeyer (1974) reported that low wavelength visible light intensified the oxidation of red OMb to brown MetMb.

American consumers have also demonstrated a definite bias against purchase of vacuum packaged beef, which displays the purple color of deoxymyoglobin (Meischen, Huffman, & Davis, 1987). It is likely that once a decision to purchase beef is made in the market, whether the beef is the red of fresh bloomed beef, the brown of discounted beef, or the purple of vacuum packaged beef, consumer eating satisfaction at home will depend only on the beef quality attributes of tenderness, juiciness, and flavor (Carpenter et al., 2001).

Cooked Color of Meat Product

Consumers use cooked color to determine whether meat is safe to eat or not (Howe et al., 1982). Fox (1996) reported three distinct pigments may commonly cause a red or pink color in cooked meats. They are: 1) undenatured myoglobin and oxymyoglobin (the red pigments of fresh meat), 2) nitrosyl hemochromes (the pink pigments of cured meat), and 3) the reduced globin hemochromes of well-cooked meats. Adequate heating of the myoglobin, oxymyoglobin, and metmyoglobin pigments in normal (pH 5.3-5.7) fresh muscle produces denatured globin ferrihemochrome, the gray pigment of cooked meat (Fox, 1966). Schmidt and Trout (1984) reported that a high pH inhibited the formation of a brown cooked meat color. The lack of brown cooked color was most visible in meat with the highest concentrations of myoglobin.

Numerous cases of food-borne illness, caused by pathogens have been attributed to inadequate cooking of meat products. It is difficult, however, to reliably verify doneness. Although USDA (1997a) has issued instructions for verifying internal temperature and cooking time for meat patties, in the absence of a thermometer, consumers tend to evaluate the degree of doneness visually, using color of ground meat as an indicator of thorough cooking. However, visual appraisal of cooked color is not always a reliable indicator (Lein, et al., 2001).

Premature Browning of Meat Product During Cooking

Inconsistent cooked beef patty color at specific internal temperatures continues to be a problem for ground beef processors, regulatory agencies, the food service industry and consumers (Berry, 1998). Thus, the USDA (1997a) changed advice for consumers to "use a meat thermometer when cooking hamburger, and do not rely on the internal color of the meat to insure food safety."

"Premature browning" (PMB) is the appearance of ground meat turning brown inside before it is sufficiently cooked. Premature browning presents an important food safety issue because patties appear fully cooked (brown) even though they have not reached an internal temperature sufficient to kill pathogens that may be present (Hague et al., 1994). Marksberry (1990) reported that some patties developed a cooked, well-done appearance at temperatures much lower than expected.

Sodeburg and Hoffman (1998) found a correlation between the raw color of uncooked ground beef and the color of cooked ground beef. This is important because the myoglobin chemistry is believed to have a strong effect upon the temperature at which the ground beef changes color. Instrumental color measurements were obtained from both the surface (external) and the interior for each package of ground beef. Instrumental color scores showed a difference in a* values suggesting a strong similarity to the differences in visual score at each temperature. This suggests that the observed color effects are due to physical/chemical differences, and are not just an observed phenomenon. The external raw instrumental color and the internal raw instrumental color were poorly correlated. No useful correlation between external raw color and the color of cooked ground beef patties were found. Patties with MetMb or OMb had more PMB than patties containing deoxymyoglobin apparently due to lower thermal denaturation temperatures for MetMb and OMb compared with deoxymyoglobin (Hunt et al., 1999).

In the Killinger et al. (2000) study, patties that are predominantly bright red and/or tan in color were more likely to be prematurely brown, whereas patties that were more purple red remained redder internally after cooking.

An examination conducted by Warren et al. (1996) observed the effects of pigment oxidation by treating ground beef with oxidizing and reducing agents. The study showed that if MetMb was in the patty interior at that time of cooking, the cooked interior color was brown; if deoxymyoglobin was present, a normal, reddish-pink cooked color developed. Warren et al. (1996) concluded that a reduced pigment was required to provide a normal cooked color change from red to brown, whereas oxidized pigments resulted in PMB of patties.

Hunt et al. (1994) indicated that the oxidative state of myoglobin at cooking time plays a major role in PMB. If there is a high level of MetMb, saturation is more likely to occur due to the reducing capacity being low, and premature browning may occur. Warren et al. (1996) suggested that reducing capacity was related to color. They found that patties with lower reducing capacity browned internally at lower temperatures than patties with a high capacity. The occurrence of PMB was also caused by the presence of OMb and

MetMb but not deoxymyoglobin in meat at the time of cooking, and the pH of the meat (Hunt et al., 1999).

Persistent Pink Phenomenon in Cooked Meat

Premature browning is not the only condition that affects the abnormal appearance of cooked meat. Ground beef cooked color scores represented all colors from red through brown when observed for all different cooking temperatures, including those temperatures less than 160°F, and this clearly shows the food safety and public health problems inherent in a "cook-until-brown-in-the-middle" message. Thus, cooking to 160°F cannot ensure that a ground beef patty will turn brown. Marksberry (1990), Van Lacck and others (1996b), Berry (1998), Soderburg and Hoffman (1998), Schoenbeck (1998), and Berry and Bigner-George (1999) observed beef patties that remained pink when cooked to 74°C or higher.

Pink color is an ever-increasing problem in patties cooked in school food service systems (USDA, 1997b). School and many other food service operations cook patties from the frozen state. Substantial pink/red cooked color has been reported in patties cooked from the frozen state and intended for inclusion in the National School Lunch Program (Van Lacck et al., 1996a). Persistent pink color also presents an important quality issue; patties still appear pink at safe internal temperatures, so consumers overcook them (Mendenhall, 1989). This hard-to-cook phenomenon seems to be related to several factors, including CO or oxide of nitrogen contamination from either gas-fired ovens (Pool, 1956) or exhaust fumes (Froning, 1983), differing concentrations of myoglobin (Froning et al., 1968), the formations of a reduced nicotinamide denatured globin hemochrome during cooking (Cornforth et al., 1986) and higher muscle pH (pH > 6.0). Furthermore, the red to pink color in fresh cooked meat can be attributed to nitric oxide (Brant, 1984), and stress (Badji et al., 1982).

These factors cause a persistent red, undercooked appearance even when cooked to internal temperatures adequate for browning of normal beef cuts (Mendenhall, 1989; Trout, 1989; Cornforth et al., 1991; Hague et al., 1994; Van Lacck et al., 1996a, 1997; and Berry, 1998).

As far back as 1958, Brown and Tappel found a consumer acceptance problem with cooked pork product having the appearance of a regenerated pink color in well-cooked roasted meat that has been refrigerated. For the consumer and the institutions who using prepared foods, including precooked frozen meats; this development of pinkness in well-cooked pork could pose a problem in acceptability of the products.

Occasionally, exposure of cooked meat to CO caused formations of globin CO hemochromes and other compounds (Cornforth, 1995; Van Lacck, et al., 1996b). When Mb is denatured, hemochromes are formed. Under reducing conditions, pink globin hemochromes may develop (Van Lacck, et al., 1996b). This undesired red or pink color may be present immediately after cooking, or it may gradually develop during distribution or during retail display. Initial pH, cooking temperature, cooking method, processing procedures, packaging, and microbial growth (growth of *Pseudomonas* in cooked bratwurst and *Clostridium* ssp. in vacuum packaged beef) have all been shown to influence pink or red color in cooked meats (Cornforth, 1991; Cornforth et al, 1995).

Several researches have attempted to identify the pigments associated with the appearance of a pink color in cooked meat. Tappel (1957) and Bernofsky et al. (1959), identified the pink pigment lying adjacent to the brown pigments of partially cooked meat as undenatured OMb. In addition, they found treating the surface of cooked meat with sodium hydrosulfite, a reducing agents, and noted the appearance of a pink color. Furthermore, he identified the pigments responsible as denatured globin hemochromes. The hemochromes oxidizes in air back to the brown hemochromes. Mendenhall (1989) and Trout (1989) suggested that myoglobin denaturation is related to pigment concentration, which suggested that initial pigment concentration indeed was related to pigment denaturation (and color). However, the correlation between Mb in the raw product and Mb percentage denaturation was only –0.57. Thus, only 30% of the variation in Mb percentage denaturation could be explained by variation in pigment concentration of the raw product. Spectral analysis of cooked products indicated that the pigment responsible for the red color was undenatured Mb.

Consumers began complaining when cooked (71°C) ground beef patties, which are normally gray inside, retain a red-pink raw color, even after cooking an additional 3-4 minutes. Furthermore, the additional cooking time removes the red, pink color but not without a concurrent loss of quality, particularly texture and juiciness (Broadbent, 1986).

The pH level plays a very important role in persistent pink in meat. Mendenhall (1989) found that red and pink color was evident to a sensory panel inside cooked ground beef patties with a pH greater than 5.7, even though a final internal temperature of 71°C was achieved. Those samples with the highest concentration of pigment exhibited the most intense color. The combined effects of Mb form and pH on cooked color development have not been reported but appear to be important to persistent pink cooked color (Hunt et al., 1999). This data of Hunt et al. (1999) indicated a more complicated nature of cooked color, because both deoxymyoglobin and oxymyoglobin had a red-pink color at 55°C color. Cornforth (1995), Mendenhall (1989), and Trout (1989, 1990) clearly indicated that myoglobin heat stability increased as pH increased, and that undenatured myoglobin may cause red color in meat cooked to 71°C if the pH is greater that 6.0. The Food Safety Inspection Service reported that if the fat content and the amount of total pigments are stable, but there are significant differences in cooked internal color then this indicates that the pH is responsible. However, when pH is held constant, the concentration of total pigment contributes to the abnormal internal color.

Another factor affecting cooked ground beef color is the amount of fat in beef patties. Low-fat beef appears to have less conduction of heat than high-fat beef. Patties can remain pink even though they have reached internal temperatures higher than the recommended 160ºF. In some cases, low fat beef patties have not only taken longer than expected to reach the targeted end-point temperature, but also maintained a pink color at temperatures of 160 to 165ºF

(Berry, 1994; Troutt et al, 1992). Several studies have shown that patties with higher fat content have shown less pink color in comparison (Berry, 1992; Van Lacck et al., 1996a; Berry, 1998).

Case Ready Packaging of Retail Meat

Retail stores are moving to more case ready products, selling 1.2 billion packages in 2000, which is more than double the number sold in 1997. Case ready meats allow retailers to order the specific cuts to suit their unique consumer demand and ethnic populations. Retailers are able to keep their meat counters stocked late at night, on holidays and through weekends. Pork and ground beef are the primary products packaged and distributed case ready. Pork products were the first case ready products to be rolled out in grocery stores. For retailers, case ready products can reduce labor cost and increase profitability. Case ready meats also reduce the liability risks to retailers should a food safety problem occur during processing in the back room. Case ready products provide a consistent product offering for the consumer from purchase to purchase (Brody and Huston, 2002).

January of 2001, in a study conducted by Cryovac Division of Sealed Air Corporation, the world's largest supplier of meat packaging equipment and services, the following store penetration estimates for the U.S. were released (note: there are more than 127,000 grocery stores in the U.S.):

- Vacuum-packaged products- 30,000 stores
- Case ready poultry- 25,000 stores

- Case ready pork- 6,000 stores
- Case ready ground beef- 10,000 stores
- Total muscle cuts- 1,000 stores
- Value-added products- 20,000 stores

Case ready products are expected to expand rapidly over the next few years. Stores using case ready products on average have experienced a 3.8% sales increase within their meat departments. Walmart cemented the case ready way of conducting business with it's knife-less backroom stance, and Pinnacle Foods made believers out of the marketplace by producing case ready products to multi-retailer specifications (Pizzico, 2002). At the IFT meeting in 2000, there was minor debate of the current U.S. case ready beef business: some speakers believed that about 10% of all beef is now delivered to retailers in case ready form, and others placed the portions higher, perhaps as much as 30%, with ground beef representing a larger share than intact cuts. Case ready meat is still not a major proportion of the beef displayed for consumers at retail level. The total retail meat industry is valued at $14 billion plus $4 billion for poultry (Brody, 2000).

To date, the cost of system management, market entrance, market sustainability and product development have clogged the path to profitability. Case ready has been a reactionary process built on market demands, which has forced cost-cutting measures at specific segments of the supply and distribution channels. For case ready to become the retailer's backroom and enhance profitability, is does not require cost lowering from packaging and equipment players. Case ready needs to become more efficient and more effective in its communications from processor, central packer, and retailer. The communications between packer and retail buyer, and between retail buyer and store meat manager, and computer to computer is what will be needed to take case ready to the next level and drive profitability (Pizzico, 2002).

Cryovac, has came up with a way to deliver a bright cherry red color at the point-of-purchase. A barrier tray is flushed with a mixture of nitrogen and carbon dioxide- two inert gases that do not affect the pigmentation of meat. Next, a lidding material (peelable barrier film) is applied to the tray to keep out oxygen. At the retailer, employees peel off the barrier portion of the film, allowing oxygen to permeate the remaining film, and thus causing meat to "bloom" and turn red. Ground beef packaged in the Cryovac system can achieve a shelf life of 16 to 18 days (Swientek, 2003).

Packages are typically given a "sell by" or "pull by" date. For tenderloin and ground beef sell by date is characteristically one day. Chuck shoulder and round cuts usually get two days of shelf life, and loin and rib cuts normally get three days of shelf life. The average value deterioration is 3.7% and 5.4% for the meat department and fresh meat case, respectively. Increasing the retail shelf life by 1-2 days to prevent this loss would save the US meat industry $175 million and about $1 billion annually (Williams et al., 1992). Package costs today are about $0.03-0.05 for conventional back-room air packaging which offers 1-3 days of shelf life; $.0.8-0.15 for high-oxygen packaging for "regional" distribution, and

$0.14-0.20 for low-oxygen packaging requiring reblooming for either regional or national distribution (Williams et al. 1992).

Carbon Monoxide

Basic Information Concerning Carbon Monoxide

Carbon monoxide (CO) is a colorless, odorless, and tasteless gas, which is produced mainly through incomplete combustion of carbon-containing materials (Sorheim et al., 1997). Carbon monoxide is also considered to be a toxic gas; therefore, its use for food packaging is not allowed in most countries. However, the Norwegian meat industry, according to Sorheim, Nissen, and Nesbakken (1997,1999), has been using a gas mixture containing 0.3-0.4% CO for the past 20 years; indeed, it is estimated that 50-60% of their retail meat and 85% of ground beef is packaged under this modified atmosphere regime (Norwegian Food Control Authority, 2001). The gas mixture consisting of 60-70% CO_2, 30-40% N_2, and 0.3-0.4% CO is used for the packaging of fresh retail meat, namely beef, pork, and lamb (Sorheim et al., 1997).

The Food and Drug Administration approved carbon monoxide as being Generally Recognized as Safe (GRAS) in 2001. It was approved at the level of 0.4% in modified atmosphere packaging (MAP). The other necessary components of the U.S. MAP system are carbon dioxide (30 percent) and nitrogen (69.6 percent). Certain regulation were also established concerning the use of CO: 1) all wood smoke, which includes CO as a component, is permitted by regulation as an ingredient in meat and poultry products under regulations issued by the U.S. Department of Agriculture (9 CFR 318.7(c) (4), 381.147 (c) (4) and 424.21 (c)); 2) Combustion product gas, which includes CO as a component at a maximum level of 4.5 percent by volume, is approved for use in the production of beverages and other foods (except fresh meat) under FDA's regulations (21 CFR 173.350); and 3) Tasteless smoke, which includes CO as a component, is the subject of GRN 000015 for use on raw tuna, before it is frozen, to preserve its taste, aroma, texture, and color. In response to GRN 000015, FDA had no questions regarding the notifier's conclusion that tasteless smoke is GRAS under the intended condition of use (USFDA, 2001).

Furthermore, several authors have pointed out that the health hazard connected with CO may have been exaggerated (El-Badawi et al., 1964; Parry, 1993). In fact, according to the International Standard for the toxicity of gases (ISO, 1995), the concentration of CO in a gas mixture necessary for reaching the toxicity limit is higher than generally thought.

The Pactive Corporation (Lake Forest, IL), the company that proposed the usage of CO in MAP packaging, did some of the initial research concerning the consumption of CO per meal. They found that assuming 30 percent of the CO present in the MAP is absorbed into the meat and there is an 85 percent reduction of CO due to the cooking of meat, thus the average consumer would only consume 0.084 milligrams of CO per meal. Further analysis calculated that worst-case intake is estimated to be 1.88 mg CO per meal; assuming that 100 percent of the CO present in MAP is absorbed into the meat and there is no reduction during cooking (USDA, 2001).

Carbon Monoxides Affect on Humans

In humans, CO binds to the iron atom of hemoglobin in red blood cells, forming carboxyhemoglobin (COHb) (Hawk et al., 1954; El-Badawi et al., 1964). Although CO acts primarily by interfering with O_2 transports, it also reduces the delivery of O_2 to the various tissues (WHO, 1987). The COHb concentration in blood, often referred to as the COHb percent is a function of the CO concentration in the air, the exposure time, and the level of physical activity of the individual (Coburn et al., 1965). It is known that carbon monoxide (CO) complexes with hemoglobin to form a bright-red pigment in human muscle tissue. Diagnostically, if this pigment is seen it is used as presumptive evidence of CO poisoning in human patients (Sax, 1951). The affinity of hemoglobin for CO is approximately 240 times higher than its affinity for O_2. CO also binds to myoglobin, cytochromes and some enzymes, but these reactions are considered to be of less importance than the formation of COHb from a human health standpoint (WHO, 1979).

The absorption and excretion of CO from the body occur relatively slowly; thus, exposure to elevated CO levels over short time periods will not result in a significant increase in the COHb level in the blood (Aronow and Isbell, 1973; Allred et al., 1989; Aunan et al., 1992). It is highly improbable that CO exposure from meat packaged in an atmosphere containing up to 0.5% will represent a toxic threat to consumers through the formation of COHb (Sorheim et al., 1997).

Very little information exists in literature on the exposure of CO from the consumption of meat that has been treated with CO gas. However, it is known that the inhalation of air containing CO at a level of 57 mg/m^3, for prolonged periods, would provide a COHb level of at least 14 times the level reached temporarily from the consumption of 225 g of meat that had been packaged in CO at the saturation level for myoglobin (Clark et al., 1976).

It is assumed that at full saturation of CO into meat myoglobin and hemoglobin the transfer from the gastrointestinal tract to the blood system would be 100%. Consequently, even for such a "worst-case" scenario, the treatment of meat with CO gas appears to contribute very little to COHb levels, relative to levels that are considered safe in the working environment. The exposure of beef to an atmosphere containing 1% CO for three consecutive days resulted in approximately 30% saturation of the meat myoglobin (Watts et al., 1978). Furthermore, the absorption of CO from the gastrointestinal tract into the blood will in all probability be less effective than the absorption of CO from the lungs, which are composed of tissues that are designed to facilitate gas exchange between the alveoli and the blood. Thus, it is highly probable that the consumption of one meal of CO-exposed meat per day will not result in a measurable increase in the COHb level in blood (Sorheim et al., 1997).

Previously, the regulatory authorities in the USA and the EU did not allow the use of CO in meat packaging. This prohibition contrasts with the fact that CO is admitted in the USA for use in vegetable processing at concentrations between 3% and 9% (Parry, 1993). Furthermore, several authors have pointed out that the health hazard associated with CO packaging systems may have been exaggerated (El-Badawi et al., 1964; Parry, 1993). In fact, according to the

International Standard for the toxicity of gases (ISO, 1995), the concentration of CO in a gas mixture necessary for reaching the toxicity limit is higher than generally thought of for the use in the meat industry.

In research conducted by Sorheim et al (1997), CO was lost from pre-saturated CO treated meat during storage in the absence of CO, with a half-life of approximately 3 days. Furthermore, Watts et al. (1978) found that the consumption of meat that had been treated for 3 days in an atmosphere containing 1% CO will yield approximately 0.1 mg of CO per kg of meat after storage and cooking at 195°F. This would be a loss of CO amounted to approximately 85%. The European Scientific Committee (2001) concluded that there are no health concerns associated with the use of 0.3%-0.5% CO in a gas mixture containing CO_2 and N_2.

Another objection that has been raised against the use of CO as a packaging gas is the potential hazard it might represent to workers in meat plants. Although the use of pure CO for mixing in the plant would certainly pose such a risk, the delivery of 1% CO in a mixture with 99% N_2, is recognized by the Norwegian meat industry as being safe handling procedure (Sorheim, 1997). Modified atmospheres containing 60-70% O_2 must also be handled carefully, because of it's explosive nature (Sorheim, 1997).

Binding Ability of Carbon Monoxide

The greatest limitation in the shelf life of meat is discoloration due to oxidation of myoglobin to metmyoglobin. In the early 1960's, a few reports were published on the use of CO as a color stabilizer in fresh meat (Williams, 1960; El-Badawi et al., 1964). In today's meat industry this discoloration can be prevented by the inclusion of a low level of CO in the gas mixture (Sorheim, 1997). The main function of low level CO in modified atmosphere packaging is to give meat a stable, cherry red lean color (Lentz, 1979). This is achieved by CO ligating to the free binding site on the Fe-atom of the heme to form the cherry red carboxymyoglobin with the structure of globin-tetrapyrrole ring- Fe^{+2} (CO). Carboxymyoglobin is more resistant to oxidation than oxymyoglobin because of the stronger binding of CO to the Fe-binding site on the myoglobin molecule (Williams, 1960; Wolfe, 1980; European Scientific Food Committee, 2001). Since these reactions do not require oxygen, treatment with CO could be a way of maintaining the desired color of prepackaged fresh beef in the absence of near absence of oxygen, thus retarding oxidative changes that affect flavor.

Carbon Monoxide Influence on Microbiological Characteristics of Fresh Meat

A possible negative aspect of using CO in MAP of retail meat is the concern that consumers might misjudge the quality of a product, because it's true microbiological status may be masked by it's stable, cherry red carboxymyoglobin lean color. Granted, consumers should be able to detect spoilage by the presence of off-odors (Kropf, 1980). This is in contrast to findings by Sorheim et al. (1997), showing that gas mixtures with CO maintains a stable, cherry red color combined with longer microbiological shelf life of the meat.

Clark et al. (1976) research has indicated that the threshold for acceptable/ unacceptable microbial contamination is approximately 7-log^{10} CFU/g aerobic plate count. The growth of psychrotropic bacteria on beef stored in MAP containing 0.5%-10% CO and N_2 was lower, relative to controls, resulting in an increase in the odor shelf life at temperatures ranging from 0-10°C. This inhibition was due to the combined result of an inhibition of the growth rate and an increase in the time of the lag phase of the bacteria system.

Clark et al. (1976), and Gee and Brown (1980 and 1981) found that atmospheres containing CO at a concentration of 5-30% have a selective action on the type of organism that grows in a mixed culture. Within their findings, they saw no effect on the growth of *Pseudomonas aeruginosa* or *P. fluorescens*, inhibited the growth rate of *Escherichia coli* (in proportion to the concentration of CO), and an increase in the lag phase of *Achromobacter*. Since the effect of CO varies with each species of bacteria tested, it seems that CO might have a selective action on organisms in a mixed culture such as those found on meat.

Enfors et al. (1979) found that aerobic and anaerobic plate counts of polyvinyl chloride (PVC) wrapped steaks reached spoilage levels ($>10^7$ cfu/cm^2) earlier than 0.5% CO-MAP, 5% CO- vacuum packaged, and 100% CO- vacuum packaged steaks. Aerobic and anaerobic plate counts of PVC ground beef reached spoilage levels by 2 weeks of storage at 2°C (Jayasingh et al., 2001). After 5 weeks of storage, the anaerobic plate count of steaks in 0.5% CO-MAP was lower than steaks in 5% CO-vacuum packaged or 100% CO-vacuum packaged, indicating more anaerobic bacterial growth in vacuum packaged than in MAP (Jayasingh et al., 2001). The lower bacterial growth in MAP than vacuum packaging was probably due to the antimicrobial effect of the CO_2 in MAP (Enfors, et al., 1979; Sorheim, 1997); due to the fact, that a concentration of 0.5% CO, seems to have no or only minor effects on bacteria and on the shelf life of meat (Clark et al., 1976; Gee and Brown, 1978; Luno et al., 1998; Sorheim, 1997).

The conclusions of The European Commission Scientific Committee (2001) stated that meat packaged in MAP containing a high concentration of CO_2 and 0.3%-0.5% CO remained microbiological safe for 11-21 days when stored at a maximum temperature of 4°C. High CO_2/ low CO gas mixtures inhibit the growth of *L. monocyteogenes*, *Y. enterocolitica* and *E. coli* 0157:H7 during storage at 4°C. This is in comparison to finding of Luno et al. (1998), who found that when using MAP of 20% CO_2/ 70% O_2/ 9% N_2, with the addition of 1% CO had no effect on the microbiological growth on ground beef and beef steaks.

Characteristics of Color Stability When Affected by Carbon Monoxide

A challenge in the MAP industry is the stabilization of the red lean color of the product (Sorheim et al., 1997). Two pigments, myoglobin and hemomyoglobin, contribute principally to the color of fresh meat. Myoglobin represents about 80-90% of the color pigment in meat, while hemomyoglobin, catalase and cytochromes contribute the remainder (European Scientific Food Committee, 2001). Many gas combinations cause substantial increases in the shelf life of meat, however, it is often limited by discoloration due to oxidation of myoglobin to metmyoglobin.

The European Scientific Food Committee (2001), Pearson (1960), Lentz (1979), and Sorheim et al. (1997, 1999) suggested that storing fresh meat under an atmosphere of CO might promote lean color stability in packaged fresh beef. The positive effect of CO on meat color was known and patented over 100 years ago (Church, 1994). During the past 20 years, the Norwegian meat industry has been using a gas mixture of 60-70% CO_2, 30-40% N_2, and 0.3-0.4% CO for the packaging of fresh retail meat, namely beef, pork, and lamb (Sorheim et al., 1997).

Discoloration of myoglobin to metmyoglobin can be prevented by the inclusion of a low level of CO in the gas mixture (Sorheim, 1997). Clark et al. (1976) found that levels of CO >0.5% resulted in a stable, red color for more than 30 days, whereas discoloration occurred after 5 days storage in control samples packaged in air. It was also found that these CO-treated samples had greater color stability than those treated in air alone. Further studies conducted by Sorheim et al. (1997, 1999) and Nissen et al. (1999) showed that meat in high CO_2/ low CO mixture have a stable bright red color for an extended period when exposed to light. Modified atmosphere packaging + CO packages received the highest color scores on an 8-point scale and maintained a bright, cherry-red color for the duration of the storage period (Kusmider et al., 2002).

At the level of 0.5% CO, treated steaks displayed lower Hunter a* values in MAP conditions after 24 hours of display than did control steaks. The CO penetration depth was 3.9 mm in 5% CO MAP compared to 1.8 mm in 0.5% CO MAP after 24 hours. Steaks and ground beef in 0.5% CO MAP maintained their desirable red lean color for the entire eight week study, but there is the possibility that after five weeks storage the product could have been spoiled but still appeared fresh (Jayasingh et al., 2001). Those results were in agreement with previous findings of Kropf (1980) concluded that CO may mask spoilage because the stable red color can last beyond the microbial shelf life of meat.

Experiments with beef containing CO, in an atmosphere of 2% CO/20% CO_2/ 78% N_2, showed meat that had a stable color; however, its color was characterized as "too artificial" by an observation panel (Renerre and Labadie, 1993). Thus based on cited literature, the presence of 0.4%-1.0% CO in MAP used for the packaging of meat seems sufficient to produce a stable, cherry red lean color without an artificial color effect (Sorheim, 1997).

Effect of Carbon Monoxide of Shelf Life Stability

One of the greatest worries of the meat industry concerning the addition of CO to MAP system is that the stable cherry lean color can last beyond the microbial shelf life of the meat and thus mask spoilage (Kropf, 1980) and under certain conditions increase the risk of growth of pathogens (Silliker and Wolfe, 1980). It was found in research conducted by Lentz (1979) and Brewer et al. (1994) that the exposure of beef to CO before vacuum packaging increased its redness and color acceptability during subsequent chilled or frozen storage.

Early research involving CO found that treating longissimus dorsi muscle with 2% CO and 98% air increased shelf life for 15 days of storage; compared to steaks packaged with air only, which only had 5-days of shelf life (El-Badawi et al., 1964).

Clark et al. (1976) found that CO at concentrations above 0.5% in gas-permeable film, increased the color shelf life over 19 days at 0°C, over 25 days at 5°C and over 28 days at 10°C. A concentration of 0.1% was too low to maintain the desired red color, and resulted in a purplish surface color similar to that obtained for samples stored in nitrogen only. Some samples were exposed to pure CO for 2-16 hours before they were packaged in air, and it was found that these CO-treated samples had no greater color stability than of those treated in air alone (Clark et al., 1976). Studies conducted by Sorheim et al. (1997, 1999) and Nissen et al. (1999) also showed that meat in a high CO_2/ low CO mixture had a stable bright red lean color. They established using a gas mixture at 4°C, meat in high CO_2/ low CO mixture displayed a stable bright red lean color longer, in fact, the shelf life in this gas mixture were 11 days for ground beef, 14 days for beef loin steaks and 21 days for pork chops, after these reported times off-odors were developed. Samples stored under high O_2 initially showed a bright red lean color of the meat, but the color was unstable and off-odors developed rapidly after storage periods of 8, 10, and 14 days, respectively. Increasing the storage temperature to 8°C. Resulting in reduced shelf life, under both conditions, to only one day for ground beef, 14 day for beef loin steaks and 21 days for pork chops.

Influence of Carbon Monoxide on Odor Shelf Life

Off-odors frequently limit the overall shelf life of meat samples. Clark and others (1976) found the odor shelf life increased with higher CO concentrations, over that of storage under nitrogen, indicated that CO directly inhibited the growth of the psychotropic bacteria used.

The odor shelf life of beef loin steaks in 0.4% CO/60% CO_2/40% N_2 was 4 days longer than steaks stored in 70% O_2/30 % CO_2 at 4°C (Sorheim et al., 1997). Other studies conducted by Luno et al. (1998) found that 1% CO/99% N_2 had an odor shelf life of 24 hours, compared with 18 days in 100% N_2, and 7 days in air at 5°C. Consequently, Kusmider et al. (2002) established that MAP + CO packaging provided the lowest amount of off-odor production over the 28 day storage period.

Carbon Monoxides Effect on Lipid Oxidation

Overall quality and consumer acceptability of meat deteriorates because of lipid oxidation and muscle pigment oxidation. Lipid oxidation rate and extent is primarily affected by polyunsaturated fatty acids content of tissue (Fennema, 1996). Ksumider et al. (2002) found that MAP containing CO showed no significant difference ($P < 0.001$) in the amount of lipid oxidation for up to 7 days but at 14 days the MAP + CO package resulted in a significantly higher ($P < 0.05$) TBA value (an indicator of increased lipid oxidation).

Bone Blackening's Affect on Marketability of Bone-in Meat

Bone blackening can negatively affect the marketability of bone-in meat at retail display. According to Gill (1996), darkened cut bone surfaces can detract from the overall appearance of a cut. Bones blackening after short periods of display is an undesirable consequences of prolonged storage for which there is no apparent means of control. Also in cut bone surfaces the hemoglobin is released from disrupted red blood cells in the marrow, which will accumulate at the surface and become dark brown and finally black when the bone is exposed to air. A study conducted Sorheim, et al. (1999) found that approximately 75% of the 30 bone-in pork loin chops used in their study, showed bone blackening in high O_2. However, the cut bones of pork chops stored in high O_2 blackened during storage, but this discoloration was not observed on bones in the CO mixture, of 60% CO_2/ 40% N_2 /0.4% CO, and in the mixture of gases (60% CO_2/ 40% N_2) with O_2 absorbers. Further studies found also found that bone blackening in pork chops was prevented by packing in a 0.4% CO mixture, probably as a result of CO binding to hemoglobin in the bone marrow (Sorheim, et al., 1999).

CHAPTER III

EXTENDING SHELF LIFE OF BEEF CUTS UTILIZING LOW LEVEL CARBON MONOXIDE IN MODIFIED ATMOSPHERE PACKAGING

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ABSTRACT

Short loins (n=14) and top sirloin butts (n=14) were obtained and aged for 14 d at Oklahoma State University Food and Agricultural Products Center. The subprimals were then over-night mailed to Cryovac Seal Air Corporation (Duncan, SC) for further processing. Twelve steaks were cut for the following procedures: lean color assessment; odor assessment; cooked internal lean color; thiobarbituric acid analysis and total plate count; and sensory panel. Steaks were then divided into one of three MAP treatments: 80% O_2/20% CO_2 (80/20), 70% N_2/ 30% CO_2 (70/30), or 69.6% N_2/ 30% CO_2/ 0.4% CO (CO). The meat trays were sealed with 1050 lidding film (Cryovac Sealed Air, Duncan, SC) for packages with the atmosphere treatment of 80/20, and Cryovac lid 550 peelable film for the atmosphere treatments of 70/30 and CO. Ground beef patties were prepared, and subjected to the same procedures and gas treatments as the steaks. Packages were then subjected to dark stored for 0, 7, or 14 d. Random steaks/patties were removed for each packaging treatment

group and were placed in retail display for 7 days at the end of each storage period. CO displayed brighter lean color ($P < 0.05$), which improved total retail display time compared to 80/20 and 70/30 for steaks and ground beef patties, especially following 7 days of dark storage. CO also reduced bone discoloration in T-bone steaks compared to gas treatments of 80/20 and 70/30. Packaging in CO did create a "hard to cook" phenomenon in ground beef patties. The patties remained persistent pink even after being cooked to 77°C (well done). The inclusion of CO also reduced lipid oxidation. Trained sensory panelists could not distinguish CO packaged products from samples in 70/30, and found them more acceptable than 80/20. Thus, CO appears to extend shelf life, while slowing rancidity, which will be more conducive to the typical distribution situation seen in the United States.

INTRODUCTION

Carbon monoxide (CO) is a colorless, odorless, and tasteless gas. CO is not allowed in meat production in many countries because it is considered a toxic gas. The Norwegian meat industry has been using a gas mixture containing 60-70% CO_2, 30-40% N_2, and 0.3-0.4% CO during the past 20 years, and it is estimated that 50-60% of retail meat is packaged this way (Sorheim et al., 1997). The Food and Drug Administration approved carbon monoxide as being Generally Recognized as Safe in 2001. In the United States, CO was approved at the level of 0.4% in modified atmosphere packaging (MAP). In order for CO to be used in MAP packaging, carbon dioxide has to be a set level of 30%, and nitrogen at the level of 69.6% (USDA, 2001).

Appearance determines how consumers perceive quality and significantly influences purchasing decisions. In the case of beef, two important visual clues that determine perceived quality are color and packaging (Issanchou, 1996). One of the greatest attributes of using CO is an increase in shelf life. In studies conducted by Sorheim et al. (1997 and 1999) and Nissen (1999), they found that CO treated MAP can remain a stable bright red color for 11 days in ground beef, 14 days in beef loin steaks, and 21 days for pork chops. This is in comparison to high O_2 packages which developed off odors and discoloration in 8, 10, and 14 days, respectfully.

The objectives of this study was to assess the impact of low-oxygen packaging systems containing CO on shelf-life of beef cuts; determine if sensory ratings are altered by a case ready system containing CO; compare effectiveness of CO versus current packaging systems on microbiological profile; and examine if CO retards the formation of metmyoglobin ("browning") and oxidative rancidity properties in case ready packaged beef cuts.

MATERIAL AND METHODS

Meat Samples

Boxed beef subprimals, short loins (IMPS # 174) and top sirloin butts (IMPS # 184), were obtained from National Beef Co. in Liberal, KS. Each identified subprimal was allowed to age for 14 days at 3°C ± 1°C in the Meat

Laboratory at the Oklahoma Food and Agriculture Products Center at Oklahoma State University. Following completion of postmortem aging, samples were over-night mailed to Cryovac Seal Air Corporation in Duncan, SC for further processing. Each short loin (n = 14) was fabricated into 12 steaks (2.54 cm thick) using a sanitized commercial band saw (Appendix A). One steak was randomly removed from each subprimal and cut in half for thiobarbituric acid analysis (TBA) and total plate count (TPC) determination, respectively. A second steak was removed and identified for a day 0 sensory panel and a third was obtained for day 0 cooked lean color panel. The day 0 TBA, TPC, sensory, and cooked lean color were considered an overall control measurement (i.e., "baseline") for analysis. The remaining steaks (n = 9) of each subprimal were individually placed in C976 foam meat trays (Cryovac- Sealed Air, Duncan, SC), and subsequently divided into three MAP treatments– 80% O_2/20% CO_2 (80/20), 70% N_2/ 30% CO_2 (70/30), or 69.6% N_2/ 30% CO_2/ 0.4% CO (CO). The meat trays were sealed with 1050 lidding film (Cryovac- Sealed Air, Duncan, SC) for the packages with the atmosphere treatment of 80/20, and Cryovac® lid 550 peelable film for the atmosphere treatments of 70/30 and CO.

Each top sirloin butt (n = 14) was fabricated into 6 steaks (2.54 cm thick), each steak was then cut in half (n=12) using the aseptic technique. One steak was removed and cut in half for TBA analysis and TPC, respectively. A second steak was removed for a day 0 sensory panel measurement and a third steak was removed for day 0 cooked lean color measurement. The day 0 TBA, TPC, sensory, and cooked lean color were considered an overall control, "baseline" steak for analysis. The remaining 9 steaks were randomly placed into C976 foam meat trays and sealed with 1050 lidding film (Cryovac Sealed Air, Duncan, SC) for the packages with the 80/20 atmosphere mixture, and Cryovac® lid 550 peelable film for the 70/30 and CO.

Chubs of 80% lean coarse ground beef (4.54 kg each) were obtained from the National Beef Case ready facility (Moultrie, GA) and ground once through a plate with 0.32 cm orifices using a Hobart 4246S Grinder (Troy, OH). After grinding and blending, patties (113 g each) were formed using a Hollymatic Super Model 54 Food Portioning patty maker (Park Forest, IL). Six patties were randomly obtained per each treatment and correspondingly divided in one-half for TBA and TPC measurement. Six trays (n = 2 patties/tray) per treatment were used for day 0 cooked color score and another 6 trays (n = 2 patties/tray) per treatment were used for day 0 sensory panel, respectfully. A total of 50 packages per treatment were made for color score analysis, each package containing two patties. The patties were packaged in C976B trays (n = 68 / modified atmosphere treatment total) and sealed with 1050 lidding film (Cryovac- Sealed Air, Duncan, SC) for the packages with the 80/20 atmosphere mixture, and Cryovac® lid 550 peelable film for the 70/30 and CO.

Packaging

Within 20 minutes of retail fabrication, meat samples were divided into one of three MAP treatments of 80/20, 70/30, or CO, respectively, and packaged using a Ross 3320 machine (Ross Industries, Inc./ Midland, VA). Samples were then packed into coolers and shipped over-night back to Oklahoma State University for further analysis.

Storage and Display

MAP packages were stored in cardboard boxes in the absence of light for 0, 7, or 14 days at 2°C ± 1°C. After the designed storage time was attained, individual packages from each treatment group were randomly removed from dark storage and placed in a commercial retail display case for 7 days under cool-white florescent light (1,600 to 1,900 lux) at 2 to 4°C. Packages were rotated randomly in the retail display case every two days. Samples were then removed and randomly divided into three equal groups for lipid oxidation measurement (i.e., TBA) and TPC, cooked color lean analysis, and sensory panel testing. This scheme was maintained for the additional storage periods (i.e., 7 and 14 days of storage).

Lean Color Assessment

Ground beef patties and the top sirloin butt steaks were visually evaluated by a six member trained panel once daily for lean color (8 = bright cherry-red, 1 = extremely dark gray), fat color (8 = creamy white, 1 = dark brown or green), percent lean discoloration (7 = 0%, 4 = 26 to 50%, 1 = 100%), and overall acceptability (7 = extremely desirable, 1 = extremely undesirable). Additionally, T-bone steaks were also assessed for all the same characteristics, with the addition of bone discoloration (7 = 0%, 4 = 26 to 50%, 1 = 100%) being evaluated. Both the *longissimus dorsi* and the *psoas major* muscles within the T-bone steaks were individually assessed. Overall acceptability represented the combined effects of lean color, fat color, and percent discoloration and was utilized as an indicator of acceptability of the retail products.

Procedures for Cooked Lean Color Analysis

MAP packages of top sirloin butt and T-bone steaks were stored in cardboard boxes in the absence of light 0, 7, or 14 days, respectively at 2°C ± 1°C. At the termination of their respective storage period, steaks were exposed to standard commercial retail display conditions and evaluated for lean color stability for 7 days. At the termination of retail display period, one third (n = ~13) of each retail cut was designated for cooked lean color analysis. Steaks were broiled on an impingement oven (Lincoln Impinger, Model 1132-00-A, Ft. Wayne, IN) at 177°C to two degrees of final doneness (i.e., 65°C and 75°C). The ground beef patties were handled according to the same described procedures prior to cooking; however, patties were cooked on a standard household grill (George Foreman Grill, Salton, Inc., Columbia, MO) for 7 to 9 minutes depending on predetermined endpoint degree of doneness: 9 minutes corresponded to well-done, 8 minutes to medium, and 7 minutes to rare.

Measurement for Cooked Color Analysis

Following cooking, steaks and ground beef patties were allowed to cool for approximately 3 minutes. Steaks and patties were then cut through their horizontal center exposing the geometric center. A trained panel then evaluated the center of each cooked lean split surface of the steaks and ground beef patties for subjective cooked color analysis. The evaluation was based on a 1 to 6 photographic scale (1 = very rare; 2 = rare; 3 = medium rare; 4 = medium; 5 = well done; and 6 = very well done) according to the Beef Steak Color Guide-Degree of Doneness chart (American Meat Science Association [AMSA]), 1995).

Objective color measurements were also taken on both split halves of the cooked sample using a Miniscan XE Plus (Hunter Lab, Reston, Virginia). L*, a*, and b* values were recorded for the geometric center of each cooked sample.

Sensory Panel

Sensory panelists were trained for sensory analysis following AMSA (1995) guidelines. Trained panelists were subjected to smelling dilute hexanal (Sigma, St. Louis, MO) to identify and establish flavors associated with rancidity. Steaks and patties were removed from their MAP treatment after their respective storage and display combination period, and were placed in vacuum packages and stored at –2°C until further analysis. Meat cuts were tempered for 24 hours at 4°C, and then broiled on an impingement oven (Lincoln Impinger, Model 1022, Ft. Wayne, IN) at 177°C to an internal temperature of 70°C (medium degree of doneness). The internal temperature was monitored using a VersaTuff 386 thermocouple thermometer (Atkins Temptec, Gainesville, FL). Six sessions, consisting of six-trained panelist/ treatment was performed. In the first two sessions, fourteen ground beef samples were evaluated per panelists; the second two sessions, eleven top sirloin butt samples were evaluated per panelists; and in the final two sessions eleven T-bone samples each were presented to each panelist. Two cubic portions (1.3 cm x 1.3 cm x cooked steak/patty thickness) from each sample were served warm to panelist under red light. The panelists were instructed to record the average of their two portions. Samples were evaluated on tenderness (8 = extremely tender; 1 = extremely tough), juiciness (8 = extremely juicy; 1 = extremely dry), cooked beef flavor (3 = strong; 1 = not detectable), off flavor (3 = strong, 1 = not detectable), and overall acceptability (7 = extremely desirable; 1 = extremely undesirable). Panelists were given unsalted crackers, distilled water, and an expectorant cup to cleanse their palate between samples.

Total Plate Count

All samples were packaged in whirl-paks and transported overnight to Food Safety Net Services (San Antonio, TX) for standard total plate counts. Food Safety Net followed standard plating methodology outlined by FDA's Bacteriological Analytical Method (BAM). Samples were diluted with peptone in a sterile stomacher bag and pummeled for 1 minute. The homogenate was then spiral plated (0.25 mL per plate in quadruplet) onto tryptic soy agar. Plates were incubated at 25°C for 48 hours, counted and reported in TPC per cm^2.

Odor Panel

Following each respective storage period and retail display, two random packages per treatment were designed for odor panel. Panelist evaluated samples on a scale of: 1 = odor not detectable; 2 = odor present, which activates smell but is not distinguishable; 3 = odor present, which activates smell, is distinguishable, not necessarily objectionable in short period; 4 = odor is present which easily activates smell, is very distinct and may be objectionable; 5 = odor present, is objectionable and may cause a person to avoid completely could cause physiological effects; 6 = odor present, which is strong, overpowering, and intolerable and easily produce physiological effects. The barrier film was cut with a knife, and the panel immediately evaluated the odor of the package.

Thiobarbituric Acid Analysis

On initial day (day 0) of the study and the final day (day 7) of each of retail display period, samples were removed from the retail display case, packaged in a whirl-pak and frozen at –20°C until further analyzed. Thiobarbituric acid (TBA) analysis was performed using the test procedures described by Buege and Aust (1978) with the following modifications: a 10 g sample was homogenized with 30 ml deionized water in a Waring Commercial Blender (Model 33BL79, Waring Products Division Dynamics Corporation of America, New Hartford, Connecticut) and centrifuged at 1850 G for 10 minutes at 4°C (Beckman Induction Drive Centrifuge, Model J-6M, Beckman Instruments, Inc., Houston, TX). Two mL of homogenate, in duplicate, were subjected to TBA reagent and cooked in a boiling water bath. After cooling, absorbencies of the supernatant at 531 nm were measured using a spectrophotometer (Beckman, Model DU 7500). Results were reported as Thiobarbituric acid reactive substance (TBARS) representing mg malondialdehyde (MDA) equivalents per kg of fresh meat.

Statistical Analyses

All results were analyzed using generalized least squares (PROC MIXED, SAS Inst., Inc., Cary, NC). Data was analyzed to measure the effect of storage time and modified atmosphere treatment on retail shelf life, sensory analysis, thiobarbituric acid analysis, cooked color analysis, and total plate count. Additional, repeated measurement analysis was performed on all color score data. All tests were conducted at the nominal significance level of 0.05.

RESULTS AND DISCUSSION

Retail Shelf Life.

T-bone Steaks.

Lean Color. Panelist scores below 4.0 were representative of unacceptable product that would have been discriminated against due to unfavorable appearance and not likely purchased by the consumer (Appendix B). Lean color ratings, assessment by the trained panel, demonstrated that over all storage periods, carbon monoxide (CO) produced T-bone steaks with more desirable ($P < 0.05$) lean color for both the *Logissimus dorsi* (LD) and *Psoas major* (PM) muscles (Tables 1 and 2). In fact, CO easily outperformed both of its MAP counterparts (70/30 and 80/20). Several findings with regard to T-bone steak lean color demonstrated the powerful tool CO can play in MAP systems. For examples, CO packaged T-bone steak LD lean color was brighter and more stable when compared to 80/20 and 70/30 counterparts. These findings were consistently observed for CO steaks stored for extended periods of time (Table 1). Both 7 and 14 day stored T-bone steaks were brighter than other MAP packaged steaks stored at identical lengths of time. In fact, CO T-bone steaks stored for 14 days were equal in lean color compared to 70/30 steaks placed into the retail case immediately following fabrication/packaging (day 0). Gill (1996) observed that muscles that have high metymoglobin activity, such as the

Logissimus dorsi, are relatively color stable in air, and their red color persists for 3 to 4 times as long as that of color unstable muscles of low metmyoglobin reducing activity, such as the *psoas major*. However, metmyoglobin reduction activity decays during the storage of muscle, so after lengthy periods of storage the color stability of initially color stable muscles is similar to that of those muscles which were initially of relatively poor color stability (Moore and Gill, 1987). CO also increased the *psoas major's* color stability (Table 2). After packaging, 80/20 and CO T-bone steaks exhibited brighter PM lean color than 70/30 counterparts. However, CO packaged T-bone steaks were brighter and maintained longer acceptability levels when compared to 7 and 14 day stored steaks in 80/20 and 70/30 MAP systems. In fact, PM muscles in 80/20 stored for 14 days were unacceptable on the initial day of retail display. Four days of acceptable retail display were achieved by PM in the CO MAP system.

Fat Color. Panelist scores below 4.0 were representative of unacceptable product that would have been discriminated against due to its unfavorable appearance and not likely purchased by consumers (Appendix B). As expected no differences ($P < 0.05$) were observed in the fat color scores of T-bone steaks, which were placed into retail display immediately following MAP (Table 3). However, a significant interaction between MAP composition and storage time on fat color of T-bone steaks was observed. After 7 days of storage, CO packaged T-bone steaks had whiter fat compared to 70/30 and 80/20 steaks. Similar trends were observed for 14 day stored steaks as both CO and 70/30 MAP T-bone steaks had more desirable fat color than the 80/20 MAP treatment. Additionally, an interaction between retail display time and case-ready atmosphere composition on T-bone steaks fat color was observed (Table 4). Compared to 80/20 and CO, fat color of T-bone steaks in 70/30 MAP became browner ($P < 0.05$) and was less stable (approximately 24 hours less acceptability). This is in agreement with previous research, which showed that oxygen depleted atmospheres demonstrated fat tissues being infiltrated with muscle pigment, which imparts initially bright pink tones to the tissue until exposure to air after rigorous exclusion of oxygen. However, tones will dull and discolor the tissue after relatively short times in air or in packs in which oxygen is added (Gill, 1996).

Lean Discoloration. Panelist scores below 4.0 were representative of unacceptable product that would have been discriminated against due to its unfavorable appearance and not likely purchased by the consumer (Appendix B). Consumers use lean color as an indication of beef freshness and will make a no-purchase decision when brown metmyoglobin reaches 30 to 40% of total pigments on the surface of fresh beef (Green, Hsin & Zipser, 1971). Table 5 reveals that CO produced less ($P < 0.05$) LD lean discoloration after day 7 of storage in comparison to its counterparts. This trend was observed when steaks were stored for 14 days. 80/20 performed much poorer in terms of lean discoloration compared to the other low oxygen MAP systems. High concentrations of O_2 caused meat to have temporary bright red lean color due to efficient oxygen binding of myoglobin to form oxymyoglobin (i.e., bright cherry-red color). However, this compound quickly oxidizes to gray-green-brown metmyoglobin (Cornforth, 1994).

Once again, early storage (day 0) appeared to benefit the 80/20 and CO MAP systems in terms of discoloration of the *psoas major* (Table 6). Both 80/20 and CO packaged T-bone steaks *psoas major* muscle was less discolored than the 70/30 treatment steaks. Actually, the MAP systems had similar *psoas major* discoloration scores (i.e., 40-50% discoloration) on display day 4 compared to initial 70/30 discoloration scores. However, as previously noticed, as storage time increased, two consistent findings occurred: 1) 80/20 MAP began to stumble in its performance and became similar to 70/30 and, 2) CO out performed the remaining MAP systems (Table 6). In summary, after 7 or 14 days of storage, less ($P < 0.05$) lean discoloration occurred on the surface of *psoas major* muscle on T-bone steaks in CO systems.

Discoloration of perishable fresh meat products is the primary basis of purchase intent. Consumers associate beef that is not bright cherry-red with unacceptability from a wholesale and freshness standpoint. It is then discounted in price, discarded, or reworked into further processed items. The ability to increase color stability and reduce discoloration from spoilage holds great profit potential (Locke, 2002).

Overall Acceptability. Panelist scores below 4.0 were representative of unacceptable product that would have been discriminated against due to its unfavorable appearance and not likely purchased by consumers (Appendix B). CO performed extremely well over the entire storage period, and is easily seen as having a very positive effect on overall desirability for both the PM muscle and the LD muscle, especially after 14 days storage (Table 7). On storage days 0 and 7, overall acceptability rating of LD in CO and 80/20 were similar ($P > 0.05$). As observed before, CO MAP steaks had excellent overall acceptability ratings regardless of storage time. The 80/20 MAP system following 14 days of storage was rated as unacceptable on display day 1. Since most retailers utilize a sell by date of 3 to 5 days for beef product, it is very important to consider a products ability to meet this demanded, especially with increased storage periods (Stubbs, 1999).

Bone discoloration. Gill (1996) is cited as saying that hemoglobin released from disrupted red blood cells in the marrow, accumulates at the surface and becomes dark brown and finally black when the bone is exposed to air. Discoloration of bone is an undesirable consequence of prolonged storage, for which, there is no apparent means of control. Panelist scores below 4.0 were representative of 26 to 50% bone discoloration and consumers would have possibly discriminated against retail cuts due to unfavorable bone appearance (Appendix B). Information summarized in Tables 8 and 9 focuses on the interaction of MAP systems and display day along with MAP and storage time, respectively. As for storage time and its influence on bone discoloration, T-bone steaks which were stored in CO exhibited retarded ($P < 0.05$) bone discoloration compared to 80/20 and 70/30 MAP steaks. Additionally, the 80/20 system promoted bone discoloration to the point of unacceptability on the initial day of display (Table 9). This is in agreement with Sorheim et al. (1999), who found that cut bone of pork chops stored in high O_2 blackened during storage, but was not observed on bones in the CO mixture.

Top Sirloin Butts.

Lean Color. Steaks packaged in 70/30, at day 0 of storage steaks were completely unacceptable for lean color, for the entire display period (Table 10). It appears that the most compromised cuts (tenderloin, top sirloin butts) benefit the most from the CO MAP system. Initially the 80/20 and CO had very cherry red lean color that was maintained throughout 5 and 6 days of display (Table 10). However, following longer storage periods, the CO system produced top sirloin steaks with stable lean colors. The 14-day stored CO sirloin steaks maintained the lean color acceptability for the entire 7-day display period.

Fat Color. For all MAP: storage time combinations, few differences were observed in fat color of top sirloin steaks (table 11). In all cases, top sirloin fat color was acceptable for 4 to 5 days of retail display.

Percent Discoloration. Once again, an unexplainable observation was seen for 70/30 treated top sirloin steak lean discoloration percentage (Table 12). Top sirloin steaks in 70/30 MAP had in excess 70% lean discoloration on the second display day of the investigation. However, this MAP treatment made a strong rebound following an additional 7-day storage period. On day 14 of storage, complete lean discoloration was noted for 80/20 top sirloin steaks, whereas minimal discoloration occurred in CO sirloin steaks.

Overall Acceptability. Overall acceptability, for the purpose of this study, is the combination attributes of lean color stability, fat browning, and lean surface discoloration. Information in Table 13 showed overall acceptability ratings for top sirloin steaks packaged in different MAP systems. Initially, 70/30 MAP top sirloin steaks received unacceptable scores. These finding are unexplainable. The 80/20 treatment groups stored for 0 or 7 days was intermediate in overall acceptability when compared to other MAP systems. Extending stored top sirloin steaks received the most desirable acceptability rating were CO packaged. In fact, following 14 days of storage, CO top sirloin steaks were capable of maintaining up to 4 days of retail display.

Ground Beef Patties.

Lean Color. As a result of the grinding process, smearing of fat particles and incorporation of oxygen within the cut matrix, ground beef is known to be very susceptible to oxidation and in turn formation of the brown pigment (i.e., metmyoglobin) is formed and observed within 24 hours of retail display. The elimination of storage and the influence of MAP of case-ready ground beef did not have any influence on lean color (Table 14). All MAP systems were capable of maintaining acceptable lean color scores for at least 5 days of retail display. The 80/20 MAP system was rated as unacceptable in terms of lean color when packages were stored for 7 or 14 days prior to retail display. Low oxygen ground beef systems (70/30 and CO) maintained lean color for 4 days of retail display following 7 days of storage. Similar findings were noticed for CO and 70/30 systems when stored for 14 days. The findings agree with Kohls and others (2001), who found color analysis of retail ground beef supported use of low oxygen treatments when a more appealing color is needed in the retail case after extended periods of storage (>7 days).

Fat Color. Marginal differences were observed for various MAP systems and their impact on fat color of ground beef patties (Table 15). All MAP systems of ground beef patties stored for 0 or 7 days exhibited acceptable fat color scores for 6 and 7 days, respectively. After 14 days of storage, all MAP ground beef systems contained acceptable fat color for 4 days of retail display.

Lean Discoloration. The CO MAP ground beef patties displayed acceptable lean discoloration rating for at least 4 days of retail display (Table 16). In conventional overwrap ground beef packaging systems, 1 day of acceptable retail case life is standard for most scenarios. In all cases in this investigation all MAP systems were capable of extending retail display times of ground beef.

Overall Acceptability. As mentioned previously, 80/20 MAP packages initially received superior overall acceptability rating after 0 days of storage.

However, after 7 or 14 days of storage, the 80/20 MAP system received unacceptable rating and were inferior to CO and 70/30 MAP systems (Table 17). Two days of acceptable retail case life was obtainable for CO and 70/30 ground beef patties following 14 days of storage.

Odor Panel.

Previous research mentioned possible deviations in odor attributes of CO case-ready meats. For the T-bone steaks, top sirloin steaks, and ground beef patties, no observed odor differences ($P > 0.05$) were noticed for various atmosphere compositions of MAP. For all cuts, storage time influenced product odor. For example, as expected, 7 day stored T-bone steaks had the mildest odor scores compared to 14 and 21 day stored T-bone steaks (Figure 1). Similarly, top sirloin steaks responded in the same manner in that 7 day stored steaks had retarded odor scores compared to 14 and 21 day stored storage product (Figure 2). For ground beef patties (Figure 3), a linear response was observed in that day-0 stored patties received the lowest, most-desirable odor ratings with the extended stored (day 21) patties having the harshest, in most cases, unacceptable odor scores. Patties stored for 7 or 14 days were intermediate to day 0, and day 21 patties for the respective odor rating

Total Plate Count.

In MAP of fresh beef, the influence of low concentrations of CO on microorganisms seem to be of either none or minor importance. Previous research show that CO incorporation in MAP systems may cause microbial "masking," due to the extended color stability of the product. However, T-bone steaks total plate counts were not influenced ($P > 0.05$) by atmospheric conditions for the various MAP systems (Table 18). For top sirloin steaks, similar trends were noticed in that very little microbial growth occurred during retail display for any of the various storage periods (Table 19). It should be mentioned that for all storage periods, CO packaged top sirloin and T-bone steaks tended to have numerically lower total plate counts compared to 70/30 and 80/20 top sirloin steaks.

Ground beef patties microbial counts varied in that more statistical differences were observed as storage time increased (Table 20). On storage day 0, significantly lower total plate counts were observed for all atmosphere treatments when compared with bacterial counts found on day 7 storage times for all modified atmospheres. On day 7 of storage, 80/20 had statistically lower TPC/g counts than ground beef patties packaged in 70/30 or CO. Researchers have concluded that CO at levels lower than 1% would probably have little to no effect on bacterial growth of meats (Clark et al., 1976; Gee and Brown, 1978; Luno et al., 1998; Sorheim, 1997). MAP containing high concentration of CO_2 typically inhibits the growth of many microorganisms (Renerre and Labadie, 1993), while N_2 has no real effect on the microbiological state of the package, and O_2 supports the growth of aerobic microorganisms (Cornforth, 1994).

Cooked Color Panel.

Persistent pinkness in cooked ground beef patties is of considerable concern for food service establishments. Consumers view ground beef patties that are pink in color in the middle of the patty as being undercooked and unsafe. In reality, these patties may be fully cooked and safe to eat.

For T-bone steaks, no evidence of persistent pinking was observed for cuts in tested modified atmosphere composition and varying storage times (Table 21). Additionally, T-bone steaks packed in 80/20 typically numerically the higher cooked color scores than steaks from other MAP systems: storage time comparisons. Conversely, top sirloin butt steaks at storage day 0 in the control MAP system were significantly different than all other storage periods and modified atmospheres (Table 22).

In 1992, the USDA recommended that ground beef should be cooked until the "juices run clear." After some investigation, USDA subsequently revised their recommendation in 1998 to cook ground beef to an internal temperature of 160°F, using a thermometer. The 1998 USDA revision on cooking recommendations was made because color is not a reliable means of determining doneness, due to cooked color abnormalities such as premature browning, persistent redness and consistent pinking. Introduction of CO into fresh meat systems have been associated with the persistent pinking phenomenon. This has been especially true when the meat item being packaged is ground beef. CO treated ground beef stored for 14 or 21 days displayed the persistent pinking abnormality when patties were thermally processed (Table 23). After 21 days of storage, CO packaged ground beef patties appeared to be pink to light red which corresponds to medium degree of doneness (Figure 4). Compared to 80/20 and 70/30, CO packaged ground beef patties were more red after cooking. It should be mentioned that not only was this seen by the trained panelist, but objective color analysis substantiated that, CO packaged patties were more pink in their internal lean color following cooking compared to 80/20 and 70/30 MAP patties.

Lipid Oxidation

According to Faustman and others (1989), the accumulation of carbonyl compounds by oxidation of unsaturated fatty acids and meat phospholipids is correlated with myoglobin oxidation in fresh beef. The highly unsaturated fatty acids and their close proximity to myoglobin in meat cause that the microsomal lipid oxidizing system to be a potentially important inductor of oxidation of myoglobin (Lin and Hultin, 1977). Greene (1971) and Renerre (1990) found that lipid oxidation and pigment oxidation in fresh meat were closely coupled. Therefore, delaying lipid oxidation should result in the consequent delay of meat discoloration. An indicator of lipid oxidation is the presence of thiobarbituric acid reactive substances (TBARS). Many research investigations have characterized meat samples having a TBARS level of 1.0 as having oxidative flavors that could be detected by trained consumer panels.

Case ready T-bone steaks did not show any effects of storage period or modified atmosphere gas composition on oxidation ($P > 0.05$) (not in tabular form); however, top sirloin butt steaks showed an atmosphere composition by storage time interaction impact on oxidative product accumulation (Table 24). CO and 70/30 packaged top sirloin steaks stored for 14 days reacted similarly in that significantly reduced TBARS values were observed. As expected, the 80/20 sirloin steaks remained statistically the same over the entire storage period and were higher compared to other MAP systems.

For ground beef patties, a general trend existed in that as storage time increased, TBARS value was elevated. The MAP system containing high oxygen levels produced ground beef patties with the highest TBARS accumulation, compared to 70/30 and CO (Figure 5). These findings agree with Brody (2000), in that low oxygen MAP system produced beef cuts with depressed and stable oxidative end products. Additionally, it has been found that MAP systems with CO reduced lipid oxidation when compared to other types of packaging (Kusmider et al. 2002).

Sensory Panel

Sensory panelists evaluated meat samples based on five categories (Appendix D). It should be noted that there were no significant main effects or interactions among any of the sensory attributes for T-bone or top sirloin butt steaks in the current investigation. Findings by Jayasingh and others (2001) stated that there were no significant sensory differences observed due to CO treatment of ground beef patties.

Furthermore, in accordance with the elevated oxidative products associated with ground beef patties stored in 80/20 MAP systems, sensory panelists detected a strong off flavor in these samples compared to 70/30 and CO treated packages (Figure 6). The low oxygen systems produced ground beef patties that regardless of storage period length, were considered to have off-flavors, which were not detectable. Patties from the 80/20 MAP system were also rated as being less desirable in the overall acceptability than 70/30 and CO treated patties (Figure 7).

IMPLICATIONS

The inclusion of low levels of carbon monoxide in modified atmosphere packaging extends the shelf life of ground beef patties, top sirloin butt and short loins steaks, especially after 7 days of dark storage. Furthermore, the inclusion of CO in MAP systems increased microbial shelf life, and had no detrimental effects on sensory characteristic. This holds great advantage for today's packaging systems, which requires typically 21 days of color stability to go from the processor, through distribution, and finally to the consumer.

Table 1. Influence of packaging atmosphere composition and storage time on lean color[a] stability of the *Logissimus dorsi* muscle in T-bone steaks.

	Storage, day		
Modified atmosphere[b]	0	7	14
70/30	5.25^{2}	4.93^{3}	4.30^{4}
80/20	5.84^{1}	4.84^{3}	3.00^{5}
CO	5.83^{1}	5.86^{1}	5.34^{2}

[a] Lean color: 8= bright cherry red; 4=moderately dark red or gray; 1= extremely dark gray.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 2. Influence of storage period, modified atmosphere composition and retail display day on the lean color[a] of the *Psoas major* muscles in T-bone steaks.

Retail display, day	Storage, day: Modified atmosphere [b]								
	0:70/30	0: 80/20	0: CO	7: 70/30	7: 80/20	7: CO	14: 70/30	14: 80/20	14: CO
1	4.71[4]	7.01[1]	6.54[1]	5.76[2]	6.04[2]	7.16[1]	5.15[3]	3.58	7.02[1]
2	4.88[4]	6.33[2]	6.11[2]	5.40[3]	5.15[3]	6.69[1]	4.75[4]	2.80	6.13[2]
3	4.44[4]	5.55[3]	5.14[3]	4.58[4]	4.49[4]	5.69[2]	4.06[5]	2.35	4.91[3]
4	4.08[5]	4.94[3]	4.70[4]	4.05[5]	3.96	5.19[2]	3.60	2.12	4.60[4]
5	3.76	4.30[4]	4.24[4]	3.84	3.26	4.92[3]	2.99	1.94	3.86
6	3.44	3.88	3.88	3.26	2.92	4.14[5]	2.82	1.99	3.38
7	3.35	3.41	3.67	2.82	2.46	3.67	2.60	1.72	3.17

[a] Lean color: 8= bright cherry red, 4=moderately dark red or gray, 1= extremely dark gray.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable lean color.

Table 3. Influence of case-ready package atmosphere composition and storage on fat color[a] stability of T-bone steaks.

	Storage, day		
Modified atmosphere[b]	0	7	14
70/30	5.14[1]	4.35[3]	4.66[2]
80/20	5.23[1]	4.40[3]	4.41[3]
CO	5.32[1]	4.69[2]	4.83[2]

[a] Fat color: 8= creamy white, 4= slightly brown, 1= dark brown or green.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 4. Effect of retail display time and case-ready atmosphere composition on fat color[a] of T-bone steaks.

	Modified atmosphere[b]		
Retail display, day	70/30	80/20	CO
1	6.70^{2}	6.50^{2}	7.04^{1}
2	6.23^{3}	5.93^{4}	6.74^{2}
3	5.07^{6}	5.11^{6}	5.43^{5}
4	4.22^{7}	4.33^{7}	4.45^{7}
5	3.96	4.00^{7}	4.05^{7}
6	3.54	3.52	3.60
7	3.29	3.36	3.31

[a] Fat color: 8= creamy white, 4= slightly brown, 1= dark brown or green.

[b] Modified atmosphere: 70/30 = 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5,6,7] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable fat color.

Table 5. Influence of case-ready atmosphere and storage period on percent lean discoloration[a] for *Logissimus dorsi* muscle in the T-bone steaks.

	Storage, day		
Modified atmosphere[b]	0	7	14
70/30	5.64^{2}	5.12^{3}	4.94^{3}
80/20	6.28^{1}	4.84^{3}	3.14^{4}
CO	6.21^{1}	5.84^{2}	5.59^{2}

[a] Percent discoloration: 1= 100%; 4=26-50%; 7= 0%.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

^{1,2,3,4} Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 6. Impact of storage period, modified atmosphere composition and retail display day on the percent lean discoloration[a] of the *Psoas major* muscles in T-bone steak.

	Storage, day: Modified atmosphere[b]								
Retail display, day	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	5.32^{2}	6.90^{1}	6.63^{1}	6.08^{2}	6.54^{1}	6.90^{1}	6.02^{2}	3.62	6.60^{1}
2	5.35^{2}	6.90^{1}	6.58^{1}	5.87^{2}	5.62^{2}	6.80^{1}	6.02^{2}	2.96	6.47^{1}
3	5.02^{3}	6.53^{1}	6.02^{2}	4.83^{3}	4.74^{3}	6.38^{1}	4.91^{3}	2.19	5.76^{2}
4	4.81^{3}	6.03^{2}	5.55^{2}	4.50^{3}	4.15^{4}	5.53^{2}	3.85	1.85	4.86^{3}
5	4.00^{4}	5.00^{3}	4.58^{3}	3.81	3.31	5.14^{3}	3.00	1.77	3.96
6	3.61	4.39^{3}	4.04^{4}	2.79	2.53	4.23^{4}	2.58	1.64	3.49
7	3.04	3.55	3.45	2.77	2.24	3.78	2.05	1.43	3.05

[a] Lean discoloration: 7= 0%; 4= 26-50%; 1= 100%.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable percentage lean discoloration.

Table 7. Influence of storage period, modified atmosphere composition and retail display day on the overall acceptability[a] of the *Logissimus dorsi* muscles in T-bone steak.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0: 80/20	0:CO	7: 70/30	7: 80/20	7: CO	14: 70/30	14:80/20	14: CO
1	5.10[3]	6.75[1]	6.49[1]	5.81[2]	6.23[1]	6.74[1]	5.04[3]	3.25	6.37[1]
2	5.44[2]	6.50[1]	6.32[1]	5.46[2]	5.46[2]	6.47[1]	4.83[3]	2.61	5.90[2]
3	5.03[3]	5.84[2]	5.84[2]	4.52[3]	3.97	5.38[2]	3.86	2.13	4.79[3]
4	4.73[3]	5.26[2]	5.29[2]	3.76	3.47	4.60[3]	3.03	1.79	3.94
5	4.40[3]	4.46[3]	4.65[3]	3.40	3.00	4.27[4]	2.77	1.71	3.31
6	3.81	4.02[4]	4.13[4]	2.97	2.49	3.60	2.18	1.49	3.00
7	3.45	3.36	3.54	2.67	2.28	3.22	2.05	1.29	2.55

[a] Overall acceptability: 7= extremely desirable; 4= acceptable; 1= extremely undesirable.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to undesirable overall acceptability.

Table 8. Effect of case-ready atmosphere and storage period on percent bone discoloration[a] in T-bone steaks.

	Storage, day		
Modified atmosphere[b]	0	7	14
70/30	3.73	3.51	3.37
80/20	3.62	3.38	3.24
CO	4.12[1]	3.69	4.15[1]

[a] Percentage bone discoloration: 1= 100%; 4= 26-50%; 7= 0%.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable percentage bone discoloration.

Table 9. Retail display day and case-ready gas compositions influence on the percentage bone discoloration[a] on T-bone steaks.

	Modified atmosphere[b]		
Retail display, day	70/30	80/20	CO
1	5.38[2]	4.31[3]	6.08[1]
2	4.04[3]	3.92	4.53[3]
3	3.75	3.58	4.00[3]
4	3.20	3.20	3.59
5	2.97	3.05	3.42
6	2.76	2.99	3.21
7	2.65	2.84	3.09

[a] Percentage bone discoloration: 1= 100%; 4=26-50%; 7= 0%.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable percentage bone discoloration.

Table 10. Influence of storage period, modified atmosphere composition and retail display day on the lean color[a] of top sirloin butt steaks.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	3.31	6.63[1]	6.60[1]	5.92[2]	5.82[2]	6.35[1]	5.55[2]	3.12	6.87[1]
2	3.67	6.08[2]	6.16[1]	5.53[2]	5.45[2]	5.79[2]	4.74[3]	2.42	6.21[1]
3	3.54	5.14[3]	5.26[3]	5.06[3]	5.14[3]	5.43[2]	4.45[4]	2.49	5.74[2]
4	3.64	4.65[3]	4.84[3]	4.35[4]	4.28[4]	4.57[3]	4.21[4]	2.25	5.40[2]
5	3.39	4.16[4]	4.57[3]	4.13[4]	3.99	4.46[4]	4.03[4]	2.20	5.06[3]
6	2.95	3.58	4.14[4]	3.67	3.57	3.96	3.67	2.14	4.56[3]
7	2.72	2.96	3.55	3.16	2.95	3.43	3.48	2.01	4.30[4]

[a] Lean color: 8= bright cherry red; 4= moderately dark red or gray; 1= extremely dark gray.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable lean color.

Table 11. Influence of storage period, modified atmosphere composition and retail display day on the fat color[a] of top sirloin butt steaks.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	6.78^{2}	7.44^{1}	7.60^{1}	6.97^{2}	6.75^{2}	6.83^{2}	6.94^{2}	5.87^{4}	7.06^{2}
2	6.50^{3}	6.85^{2}	7.05^{2}	6.38^{3}	6.11^{4}	6.40^{3}	6.22^{3}	5.12^{5}	6.44^{3}
3	5.97^{4}	5.90^{4}	6.39^{3}	5.38^{5}	5.20^{5}	5.27^{5}	5.03^{5}	4.60^{6}	5.20^{5}
4	4.95^{6}	4.88^{6}	5.25^{5}	4.01^{8}	3.83	3.95	4.21^{7}	4.29^{7}	4.38^{7}
5	4.43^{7}	4.05^{8}	4.45^{7}	3.79	3.66	3.65	3.63	3.88	3.79
6	3.94	3.50	3.93	3.43	3.18	3.36	3.64	3.50	3.61
7	3.47	3.03	3.28	3.00	2.76	2.86	3.43	3.69	3.44

[a] Fat color: 8= creamy white; 4= slightly brown; 1= dark brown or green.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5,6,7,8] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable fat color.

Table 12. Impact of storage period, modified atmosphere composition and retail display day on the percentage lean discoloration[a] of top sirloin butt steaks.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	4.08^{4}	6.92^{1}	6.73^{1}	6.56^{1}	6.62^{1}	6.56^{1}	6.56^{1}	3.62	6.65^{1}
2	3.77	6.59^{1}	6.54^{1}	6.27^{1}	6.29^{1}	6.24^{1}	6.03^{2}	2.93	6.61^{1}
3	3.82	6.33^{1}	6.33^{1}	5.58^{2}	5.61^{2}	5.63^{2}	5.31^{2}	2.69	5.96^{2}
4	3.96	5.60^{2}	5.57^{2}	5.01^{3}	4.77^{3}	5.07^{3}	4.48^{3}	2.20	5.58^{2}
5	3.30	4.48^{3}	4.65^{3}	4.39^{3}	4.18^{4}	4.55^{3}	3.98	1.96	5.13^{3}
6	2.99	3.92	4.45^{3}	3.83	3.64	4.09^{4}	3.78	1.94	4.44^{3}
7	3.64	3.16	3.67	3.35	3.20	3.69	3.78	1.81	4.56^{3}

[a] Percentage discoloration: 7= 0%; 4= 26-50%; 1= 100%.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable percentage lean discoloration.

Table 13. Influence of storage period, modified atmosphere composition and retail display day on the overall acceptability[a] of top sirloin butt steaks.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	2.63	6.61[1]	6.45[1]	5.67[2]	5.58[2]	5.89[2]	5.61[2]	2.63	6.35[1]
2	2.87	5.92[1]	5.82[2]	4.98[3]	4.89[3]	5.02[3]	4.86[3]	1.90	5.99[1]
3	2.80	4.89[3]	4.97[3]	4.33[3]	4.24[4]	4.41[3]	3.72	1.75	4.77[3]
4	2.73	3.96	4.20[4]	3.32	3.08	3.46	3.16	1.58	4.21[4]
5	2.16	3.04	3.26	3.04	2.94	3.33	2.77	1.43	3.43
6	2.07	2.55	2.89	2.58	2.44	2.74	2.36	1.35	2.89
7	1.63	1.79	2.01	2.37	2.14	2.54	2.12	1.30	2.57

[a] Overall acceptability: 7=extremely desirable; 4= acceptable; 1= extremely undesirable.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable overall acceptability.

Table 14. Influence of storage period, modified atmosphere composition and retail display day on the lean color[a] of ground beef patties.

	Storage, day: Modified atmosphere[b]								
Retail display, day	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7: CO	14:70/30	14:80/20	14:CO
1	5.86^{5}	7.56^{1}	7.45^{2}	6.93^{3}	3.98	7.83^{1}	6.92^{3}	3.41	7.43^{2}
2	5.66^{5}	7.44^{2}	7.14^{2}	6.72^{3}	3.91	7.68^{1}	5.05^{7}	2.87	6.81^{3}
3	4.83^{7}	6.55^{4}	6.01^{5}	5.75^{5}	3.25	6.36^{4}	3.54	3.01	4.59^{8}
4	4.62^{8}	5.41^{6}	5.29^{6}	4.25^{9}	2.84	4.54^{8}	2.71	2.83	3.29
5	4.18^{9}	4.63^{8}	4.55^{8}	3.81	3.01	3.83	2.64	2.70	2.79
6	3.47	3.80	3.83	3.20	3.00	3.17	1.89	2.75	1.86
7	2.09	2.35	2.10	2.79	2.81	2.80	1.73	2.76	1.74

[a] Lean color: 8= bright cherry red; 4= moderately dark red or gray; 1= extremely dark gray.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5,6,7,8,9] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable lean.

Table 15. Influence of storage period, modified atmosphere composition and retail display day on the fat color[a] of ground beef patties.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0: 80/20	0/CO	7: 70/30	7: 80/20	7: CO	14: 70/30	14:80/20	14: CO
1	6.59^{5}	7.06^{2}	7.03^{3}	7.21^{2}	5.88^{8}	7.40^{1}	6.98^{3}	5.23^{10}	7.13^{2}
2	6.15^{7}	6.80^{4}	6.78^{4}	6.79^{4}	5.54^{9}	6.96^{3}	5.39^{10}	4.81^{12}	6.40^{6}
3	6.08^{7}	6.66^{4}	6.47^{5}	6.02^{7}	5.21^{10}	6.31^{6}	4.80^{12}	4.98^{11}	5.08^{11}
4	5.35^{10}	5.73^{8}	5.52^{9}	5.37^{10}	5.00^{11}	5.60^{9}	4.13^{15}	4.17^{15}	4.39^{14}
5	4.48^{13}	4.93^{11}	4.63^{12}	4.76^{12}	4.55^{13}	4.93^{11}	3.75	3.91	3.75
6	4.02^{15}	4.26^{14}	4.15^{15}	4.74^{12}	4.75^{12}	4.69^{12}	3.63	3.74	3.67
7	3.28	3.45	3.28	4.34^{14}	4.41^{14}	4.31^{14}	3.60	3.92	3.60

[a] Fat color: 8= creamy white; 4= slightly brown; 1= dark brown or green.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable fat color stability.

Table 16. Influence of storage period, modified atmosphere composition and retail display day on the percentage lean discoloration[a] of ground beef patties.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	6.25^{2}	7.00^{1}	7.00^{1}	6.78^{1}	4.61^{6}	6.92^{1}	6.87^{1}	6.17^{3}	7.00^{1}
2	5.94^{3}	7.00^{1}	6.95^{1}	6.48^{2}	4.33^{7}	6.92^{1}	5.54^{4}	4.05^{7}	6.50^{2}
3	5.72^{4}	6.71^{1}	6.36^{2}	5.16^{5}	3.91	5.73^{4}	3.90	3.62	4.70^{6}
4	4.92^{5}	5.80^{4}	5.15^{5}	3.87	2.61	4.00^{7}	2.26	1.97	2.83
5	3.63	4.80^{6}	3.70	3.04	2.45	3.10	2.29	2.08	2.35
6	3.22	3.59	3.28	2.30	2.18	2.34	1.00	1.00	1.00
7	1.38	1.66	1.44	1.75	1.82	1.79	1.00	1.00	1.00

[a] Percentage discoloration: 7= 0%; 4= 26-50%; 1= 100%.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5,6,7] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable percentage lean discoloration.

Table 17. Influence of storage period, modified atmosphere composition and retail display day on the overall acceptability[a] of ground beef patties.

Retail display, day	Storage, day: Modified atmosphere[b]								
	0:70/30	0: 80/20	0/CO	7: 70/30	7: 80/20	7: CO	14: 70/30	14:80/20	14: CO
1	5.16^{5}	6.78^{1}	6.74^{1}	6.67^{1}	4.24^{7}	6.95^{1}	6.52^{2}	2.1	6.67^{1}
2	5.23^{5}	6.64^{2}	6.64^{2}	6.27^{3}	3.56	6.91^{1}	4.29^{7}	1.90	6.13^{3}
3	4.34^{7}	5.85^{4}	5.42^{5}	4.36^{7}	2.51	5.07^{6}	2.07	1.52	3.31
4	2.92	4.18^{7}	3.07	2.39	1.64	3.02	1.34	1.26	1.82
5	2.89	3.73	2.94	2.33	1.76	2.41	1.22	1.04	1.28
6	2.55	2.93	2.54	1.59	1.50	1.58	1.00	1.00	1.00
7	1.07	1.34	1.09	1.11	1.17	1.11	1.00	1.00	1.00

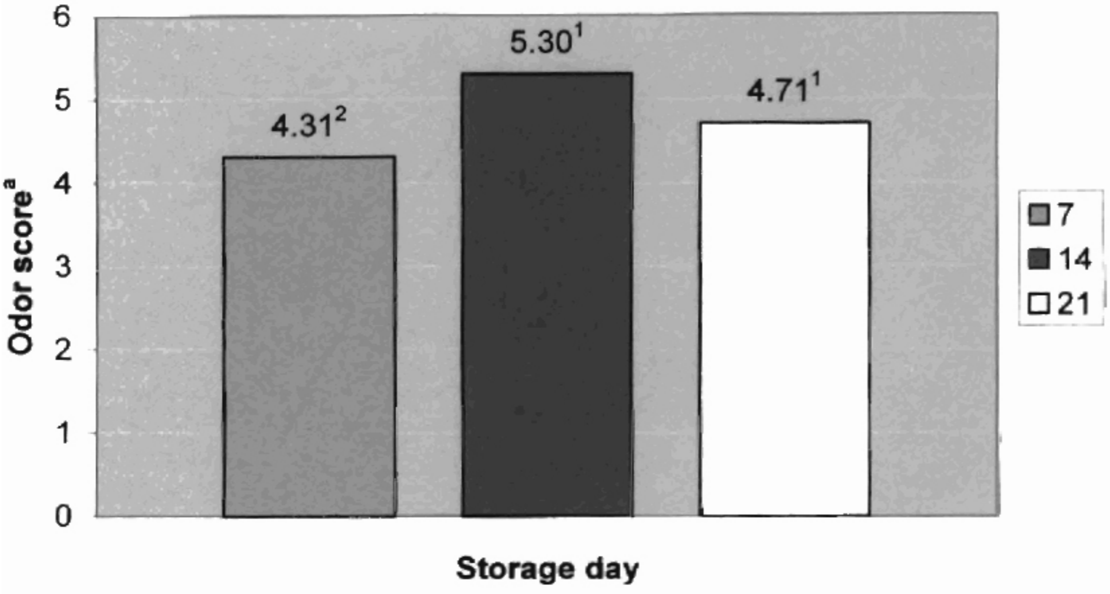
[a] Overall acceptability: 7=extremely desirable; 4= acceptable; 1= extremely undesirable.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4,5,6,7] Means that do not share a common superscript are significantly different ($P < 0.05$).

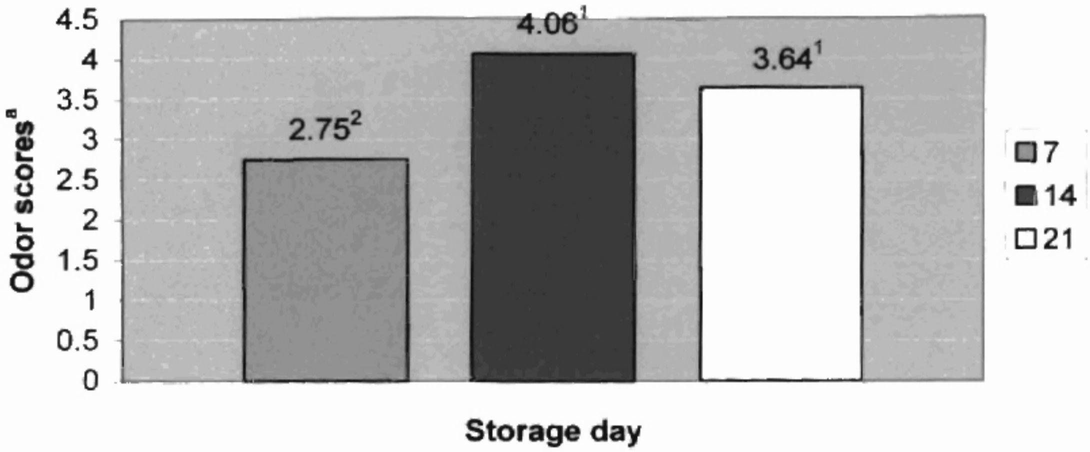
General note: Shaded area corresponds to unacceptable overall acceptability.

Figure 1: The main effect of day on odor panelist scores for case ready T-bone steaks.



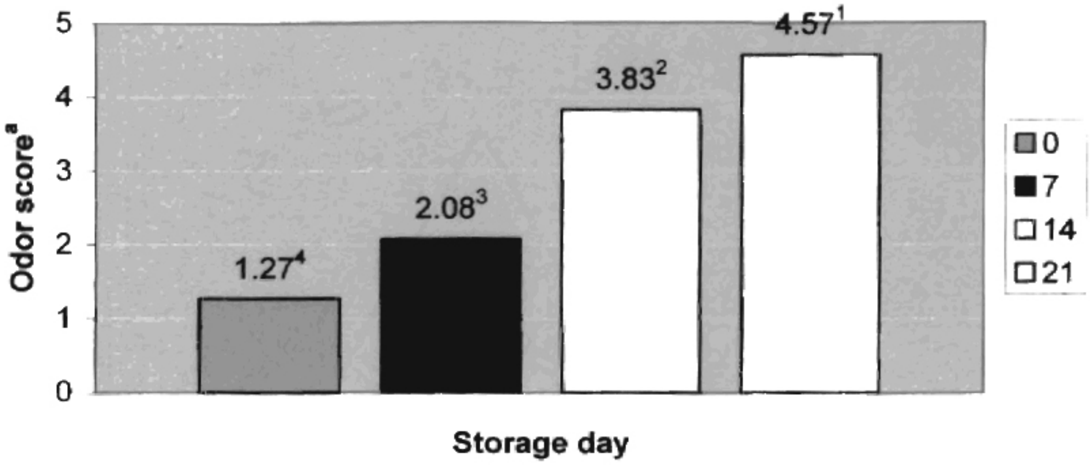
[a] 1= odor not detectable; 4= odor is present which easily actives smell, is distinct and may be objectionable; 6= odor present, which is strong, overpowering, and intolerable and easily produce physiological effects.
[1,2] Means that do not share common superscript are significantly different ($P < 0.05$).

Figure 2: The main effect of day on odor panelist score for top sirloin butt steaks.



[a] 1= odor not detectable; 4= odor is present which easily actives smell, is distinct and may be objectionable; 6= odor present, which is strong, overpowering, and intolerable and easily produce physiological effects.
[1,2] Means that do not share common superscript are significantly different ($P < 0.05$).

Figure 3: The influence on the main effect of day on odor panelist scores for ground beef patties.



[a] 1= odor not detectable; 4= odor is present which easily actives smell, is distinct and may be objectionable; 6= odor present, which is strong, overpowering, and intolerable and easily produce physiological effects.

[1,2,3,4] Means that do not share common superscript are significantly different ($P < 0.05$).

Table 18. The influence of modified atmosphere composition on total plate counts (10^3TPC/g) of case-ready T-bone steaks.

	Storage, day			
Modified atmosphere[a]	0	7	14	21
Con	9.99[1]			
70/30		12.60[2]	12.22[2]	14.10[2]
80/20		12.43[2]	13.15[2]	13.02[2]
CO		11.29[2]	11.72[2]	12.05[2]

[a] Modified atmosphere: Con= overall control; 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.
[1,2] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 19. The influence of modified atmosphere composition and storage day on total plate counts (10^3TPC/g) of case-ready top sirloin butt steaks.

	Storage, day			
Modified atmosphere[a]	0	7	14	21
Con	4.48[1]			
70/30		11.21[2]	11.70[2]	12.84[2]
80/20		11.66[2]	12.70[2]	12.71[2]
CO		10.88[2]	10.28[2]	10.59[2]

[a] Modified atmosphere: Con= overall control; 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 20. The influence of modified atmosphere composition and storage day on total plate count (10^3TPC/g) for case ready ground beef patties.

	Modified atmosphere[a]		
Storage, day	70/30	80/20	CO
0	8.37[3]	7.78[3]	8.40[3]
7	12.26[1]	10.20[2]	11.59[1]
14	12.85[1]	12.81[1]	12.95[1]
21	12.50[1]	12.29[1]	13.10[1]

[a] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 21. The effect of modified atmosphere composition and storage day on the cooked color score[a] of case-ready T-bone steaks.

Modified atmosphere[b]	Storage, day			
	0	7	14	21
Con	4.43			
70/30		4.50	4.58	4.30
80/20		4.08	4.25	3.82
CO		4.56	4.25	4.16

[a] Cooked color score: 1= very rare color, 2= rare color, 3= medium rare color, 4= medium color, 5= well done color, 6= very well done color.
[b] Modified atmosphere: Con= overall control 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

Table 22. The effect of modified atmosphere composition and storage day on the cooked color score[a] of top sirloin butt steaks.

	Storage, day			
Modified atmosphere[b]	0	7	14	21
Con	4.39[1]			
70/30		5.07[2]	4.03[2]	5.90[2]
80/20		5.70[2]	5.28[2]	5.28[2]
CO		5.23[2]	4.48[2]	4.93[2]

[a] Cooked color score: 1= very rare color, 2= rare color, 3= medium rare color, 4= medium color, 5= well done color, 6= very well done color.

[b] Modified atmosphere: Con= overall control; 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 23. The effect of modified atmosphere composition and storage day on the cooked color score[a] of ground beef patties.

	Storage, day			
Modified atmosphere[b]	0	7	14	21
70/30	5.26^{2}	5.93^{1}	5.79^{1}	5.18^{2}
80/20	6.00^{1}	6.00^{1}	5.85^{1}	5.19^{2}
CO	5.90^{1}	6.00^{1}	5.23^{2}	4.17^{3}

[a] 1= very rare color, 2= rare color, 3= medium rare color, 4= medium color, 5= well done color, 6= very well done color.

[b] Modified atmosphere: 70/30= 70% N2/ 30% CO2; 80/20= 80% O2/ 20% CO2; CO= 69.6% N2/ 30% CO2/ 0.4% CO.

[1,2,3] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table 24. Influence of packaging atmosphere composition and storage time on oxidative properties (TBA, mg MDA/kg sample) of top sirloin butt steaks.

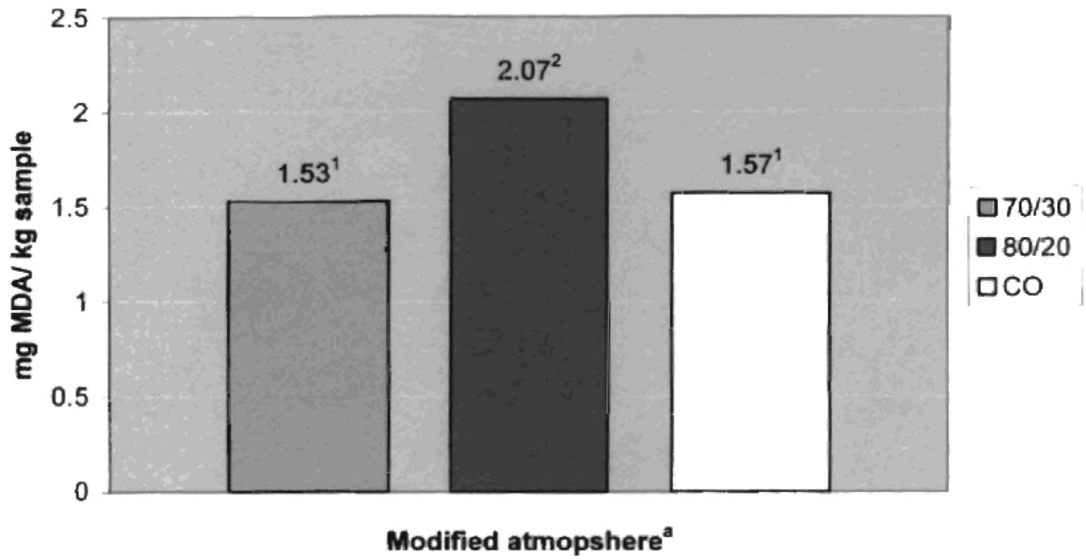
	Storage, day			
Modified atmosphere[a]	0	7	14	21
Con	0.71[2]			
70/30		1.82[1]	0.82[2]	1.40[2]
80/20		4.11[1]	2.92[1]	3.08[1]
CO		2.75[1]	0.83[2]	0.64[2]

[a] Modified atmosphere: Con= overall control, 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2] Means that do not share a common superscript are significantly different ($P < 0.05$).

Figure 4. The effect of MAP packaging in CO on cooked lean color in ground beef patties.

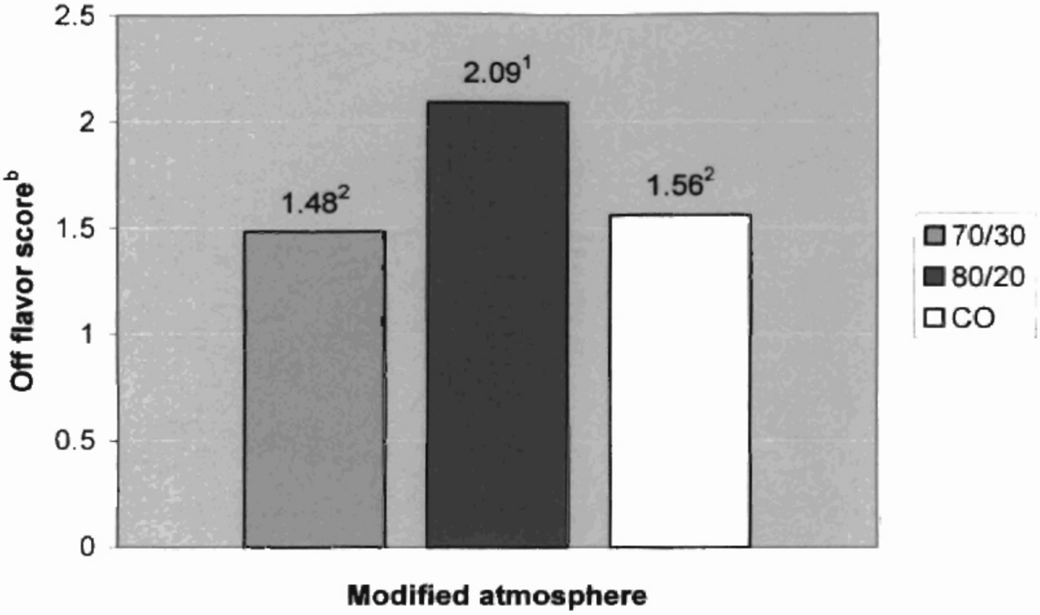
Figure 5: The main effect of modified atmosphere on oxidative properties (TBA) of ground beef patties.



[a] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30 CO_2/ 0.4% CO.

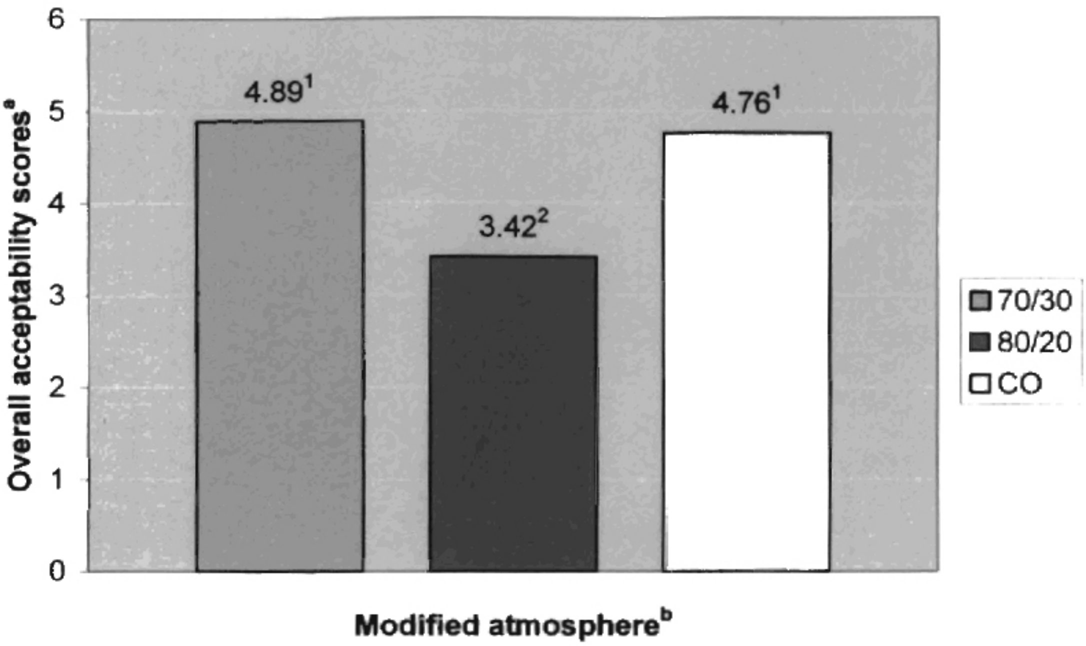
[1,2] Means that do not share common superscript are significantly different ($P < 0.05$).

Figure 6: The effect of modified atmosphere composition[a] on off flavors in ground beef patties



[a] Off flavor score: 3= strong; 2= slightly detectable; 1= not detectable.
[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30 CO_2/ 0.4% CO.
[1,2] Means that do not share common superscript are significantly different ($P < 0.05$).

Figure 7: The effect of modified atmosphere composition on overall acceptability in ground beef patties



[a] Overall acceptability: 7= extremely desirable; 4= acceptable; 1= extremely undesirable.
[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30 CO_2/ 0.4% CO.
[1,2] Means that do not share common superscript are significantly different ($P < 0.05$).

APPENDIX

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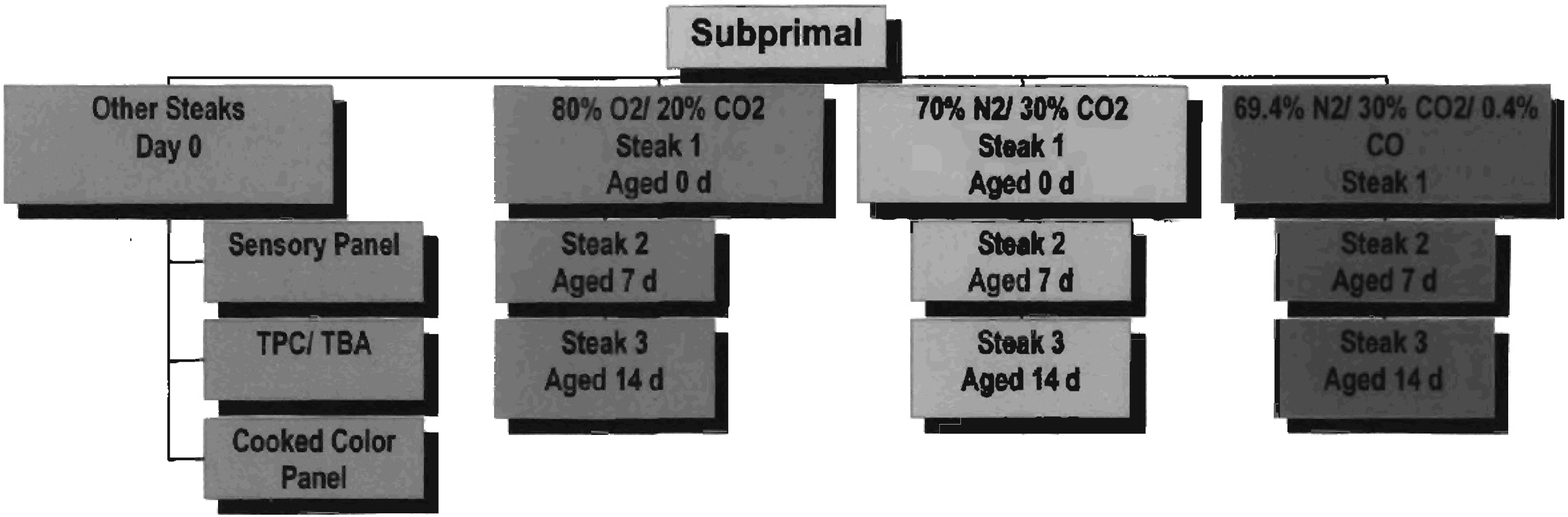
APPENDIX FIGURES

APPENDIX A

Project Design



Appendix B

COLOR SCORE CRITERIA

Lean Color
8 = Bright Cherry Red
7 = Moderately Bright Cherry Red
6 = Cherry Red
5 = Slightly Dark Red
4 = Moderately Dark Red or Gray
3 = Dark Red or Gray
2 = Very Dark Gray
1 = Extremely Dark Gray

Fat Color
8 = Creamy White
7 = Mostly Creamy White
6 = Slightly Tan
5 = Tan
4 = Slightly Brown
3 = Moderately Brown
2 = Brown or Slightly Green
1 = Dark Brown or Green

Lean Discolor (% Browning)
7 = 0%
6 = 1-10%
5 = 11-25%
4 = 26-50%
3 = 51-75%
2 = 76-99%
1 = 100%

Overall Desirable
7 = Extremely Desirable
6 = Desirable
5 = Slightly Desirable
4 = Acceptable
3 = Slightly Undesirable
2 = Undesirable
1 = Extremely Undesirable

Bone Discoloration (% Blackening)
7 = 0%
6 = 1-10%
5 = 11-25%
4 = 26-50%
3 = 51-75%
2 = 76-99%
1 = 100%

Appendix C

Odor Panel for CO Project

Sample #	Objectionable Category
1	
2	
3	
4	
5	
6	
7	
8	
9	

Objectionable Categories:

1. Odor not detectable
2. Odor present, which activates smell but is not distinguishable
3. Odor present, which activates smell, is distinguishable, not necessarily objectionable in short periods
4. Odor is present which easily activates smell, is very distinct and may be objectionable
5. Odor present, is objectionable and may cause a person to avoid completely could cause physiological effects
6. Odor present, which is strong, overpowering, and intolerable and easily produce physiological effects

Appendix D

SENSORY BALLOT

Booth # ______ **Date:** ______ **Time:** ______ **Name:** ______

Sample	Tenderness	Juiciness	Cooked Beef Flavor	Off Flavor	Comment	Overall Acceptability
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						

Tenderness

8 Extremely Tender
7 Very Tender
6 Moderately Tender
5 Slightly Tender
4 Slightly Tough
3 Moderately Tough
2 Very Tough
1 Extremely Tough

Juiciness

8 Extremely Juicy
7 Very Juicy
6 Moderately Juicy
5 Slightly Juicy
4 Slightly Dry
3 Moderately Dry
2 Very Dry
1 Extremely Dry

Overall Acceptability

7 Extremely Desirable
6 Desirable
5 Slightly Desirable
4 Acceptable
3 Slightly Undesirable
2 Undesirable
1 Extremely Undesirable

Cooked Beef Flavor

3 Strong
2 Slightly Detectable
1 Not Detectable

Off Flavor

3 Strong
2 Slightly Detectable
1 Not Detectable

Table A. Effect of retail display day in relationship to storage on the lean color[a] of *Logissimus dorsi* muscle from case-ready T-bone steaks.

	Storage, day		
Retail display, day	0	7	14
1	6.33^{1}	6.56^{1}	5.51^{2}
2	6.27^{1}	6.21^{1}	4.92^{2}
3	6.04^{1}	5.50^{2}	4.30^{3}
4	5.60^{2}	5.06^{2}	4.18^{3}
5	5.31^{2}	4.89^{2}	3.86^{3}
6	5.13^{2}	4.24^{3}	3.52^{3}
7	4.80^{2}	4.00^{3}	3.35^{3}

[a] Lean color: 8= bright cherry red, 4=moderately dark red or gray, 1= extremely dark gray.

[1,2,3] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table B. Retail display day and storage time influence on fat color[a] stability for T-bone steaks.

Retail display, day	Storage, day 0	Storage, day 7	Storage, day 14
1	7.10[1]	6.77[2]	6.37[3]
2	6.85[2]	6.15[3]	5.90[4]
3	5.74[4]	4.89[4]	4.98[5]
4	4.93[5]	3.73	4.34[6]
5	4.47[6]	3.64	3.91
6	3.93	3.19	3.54
7	3.60	2.99	3.38

[a] Fat color: 8= creamy white, 4= slightly brown, 1= dark brown or green.

[1,2,3,4,5,6] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable fat color.

Table C. Effect of storage period, modified atmosphere composition and retail display day on the overall acceptability [a] of the *Psoas major* muscles in T-bone steak.

	Storage, day: Modified atmosphere[b]								
Retail display, day	0:70/30	0:80/20	0:CO	7:70/30	7:80/20	7:CO	14:70/30	14:80/20	14:CO
1	4.70^{3}	6.54^{1}	6.37^{1}	5.67^{2}	5.91^{2}	6.73^{1}	4.90^{3}	2.58	6.37^{1}
2	4.60^{3}	6.16^{1}	5.89^{2}	5.23^{2}	4.96^{3}	6.37^{1}	4.59^{3}	1.99	5.75^{2}
3	4.25^{4}	5.47^{2}	5.31^{2}	3.92	3.65	5.20^{3}	3.37	1.62	4.31^{4}
4	3.75	4.57^{3}	4.43^{4}	3.22	2.88	4.38^{4}	2.46	1.35	3.42
5	3.26	3.75	3.72	2.92	2.58	3.89	2.03	1.22	2.72
6	2.85	3.19	3.21	2.32	2.02	3.16	1.79	1.18	2.50
7	2.45	2.69	2.68	2.04	1.72	2.85	1.49	1.14	2.09

[a] Overall acceptability: 7= extremely desirable; 4= acceptable; 1= extremely undesirable.

[b] Storage, day: Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

General note: Shaded area corresponds to unacceptable overall acceptability.

Table D. The influence of modified atmosphere composition and storage day on drip loss percentage[a] for T-bone steaks.

	Modified atmosphere[b]		
Storage, day	70/30	80/20	CO
7	1.71	1.70	1.70
14	1.87	1.95	2.25
21	2.06	2.40	1.83

[a] Drip loss percentage equation: [(initial wgh - final wgh) / initial] * 100

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

Table E. The effect of modified atmosphere composition and storage day on drip loss percentage[a] for top butt steaks.

	Modified atmosphere[b]		
Storage, day	70/30	80/20	CO
7	3.11	2.89	2.28
14	3.81	4.44	3.94
21	3.29	4.07	3.57

[a] Drip loss percentage equation: [(initial wgh - final wgh) / initial] * 100

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

Table F. The influence of modified atmosphere composition and storage day on the L* value[a] for ground beef patties.

	Modified atmosphere[b]		
Storage, day	70/30	80/20	CO
0	53.86^{1}	47.68^{2}	53.11^{1}
7	46.68^{2}	44.64^{2}	44.98^{2}
14	53.24^{1}	54.06^{1}	52.80^{1}
21	52.41^{1}	55.77^{1}	52.68^{1}

[a] Represents light reflection white to black.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table G. The influence of modified atmosphere composition and storage day on the a* value[a] for ground beef patties.

	Modified atmosphere[b]		
Storage, day	70/30	80/20	CO
0	7.23[1]	6.15[2]	6.32[2]
7	7.63[1]	7.19[1]	7.71[1]
14	5.79[2]	5.42[3]	6.54[2]
21	6.26[2]	3.58[3]	7.08[1]

[a] Represents light reflection red to green.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

[1,2,3] Means that do not share a common superscript are significantly different ($P < 0.05$).

Table H. The influence of modified atmosphere composition and storage day on the b^* value[a] for ground beef patties.

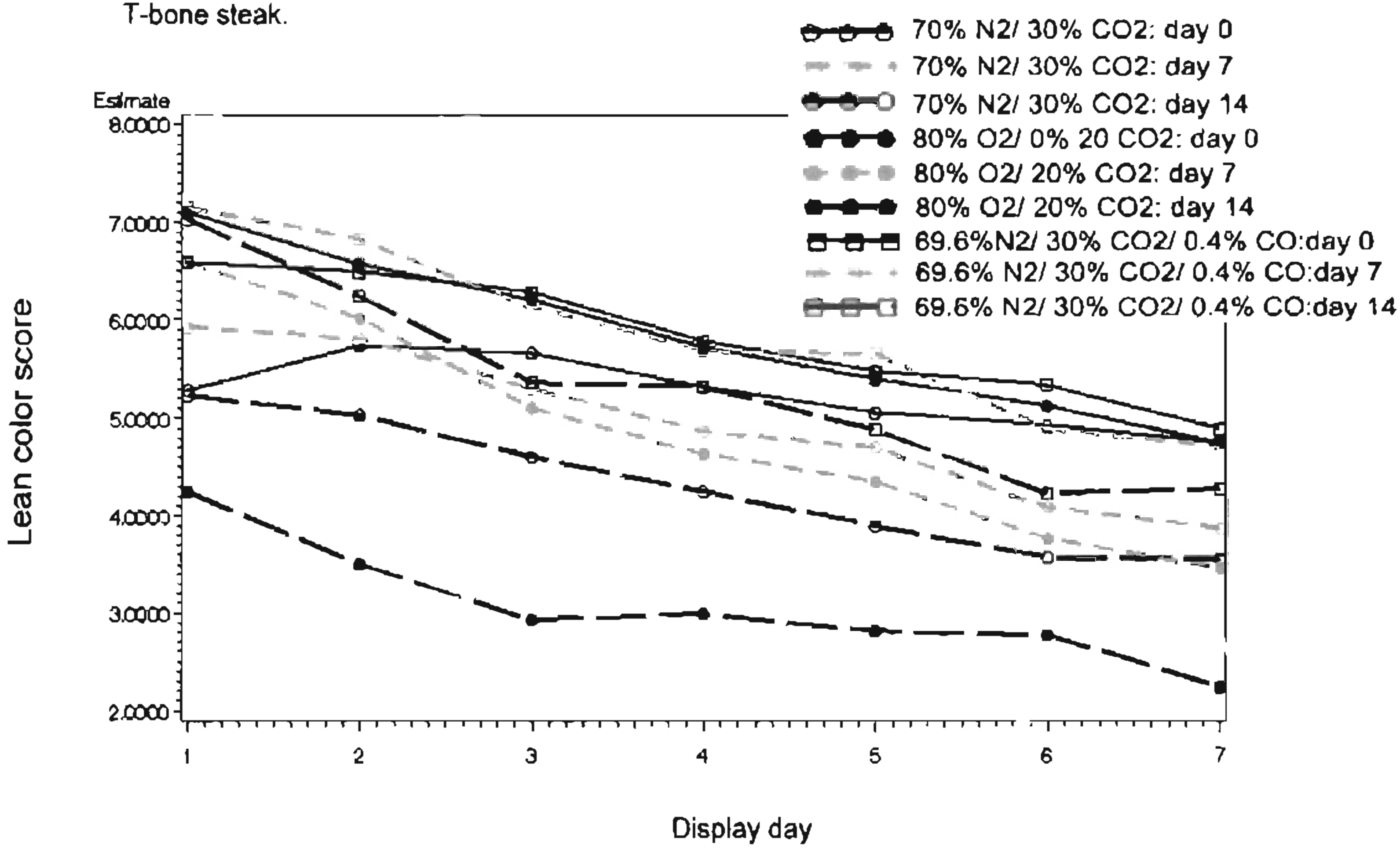
	Modified atmosphere[b]		
Storage, day	70/30	80/20	CO
0	18.67^{1}	17.88^{1}	18.32^{1}
7	17.58^{2}	19.00^{1}	16.82^{2}
14	14.27^{4}	15.71^{3}	14.48^{3}
21	15.58^{3}	16.88^{2}	15.14^{3}

[a] Represents light reflection blue to yellow.

[b] Modified atmosphere: 70/30= 70% N_2/ 30% CO_2; 80/20= 80% O_2/ 20% CO_2; CO= 69.6% N_2/ 30% CO_2/ 0.4% CO.

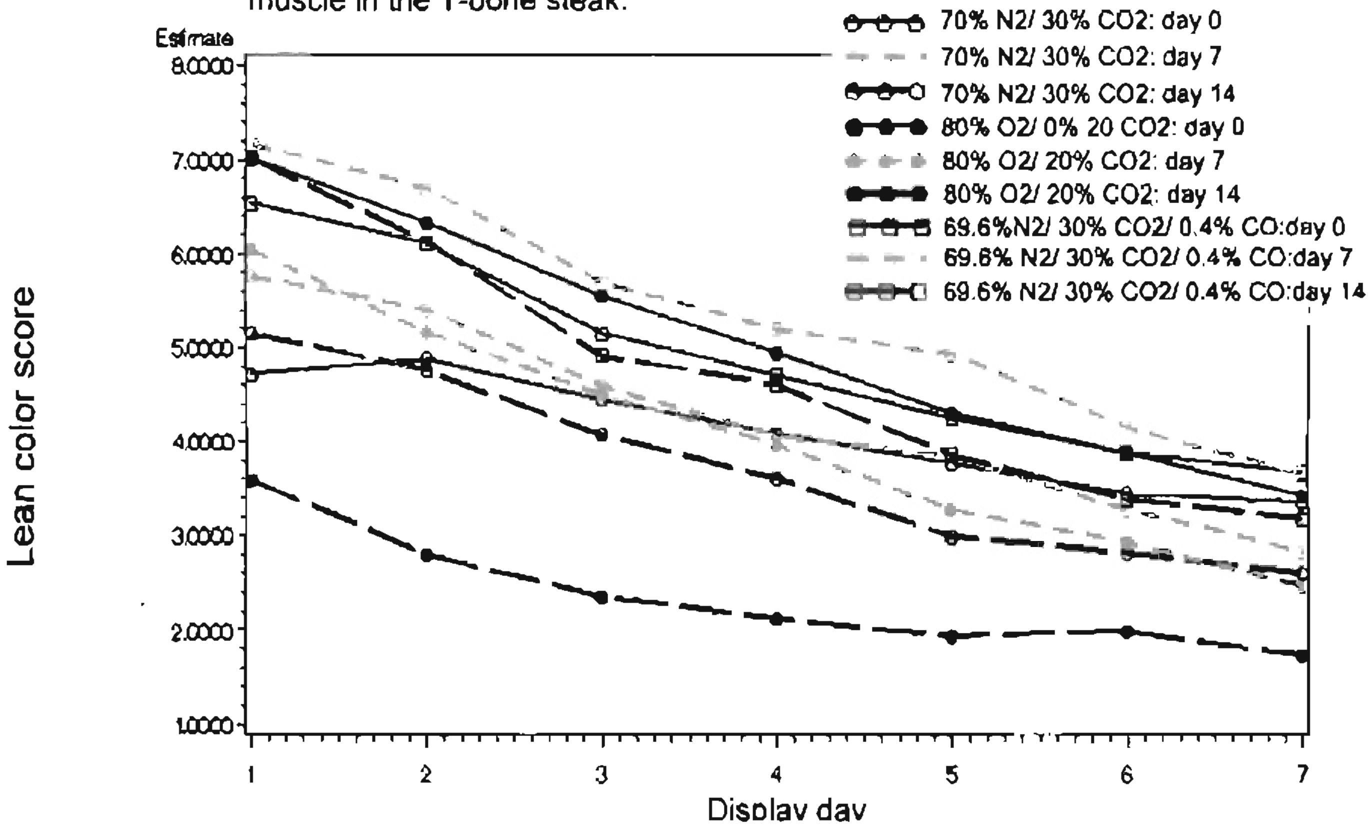
[1,2,3,4] Means that do not share a common superscript are significantly different ($P < 0.05$).

Figure A. Influence of storage period, modified atmosphere composition, and display day on the lean color[a] of the *Longissimus dorsi* muscle in the T-bone steak.



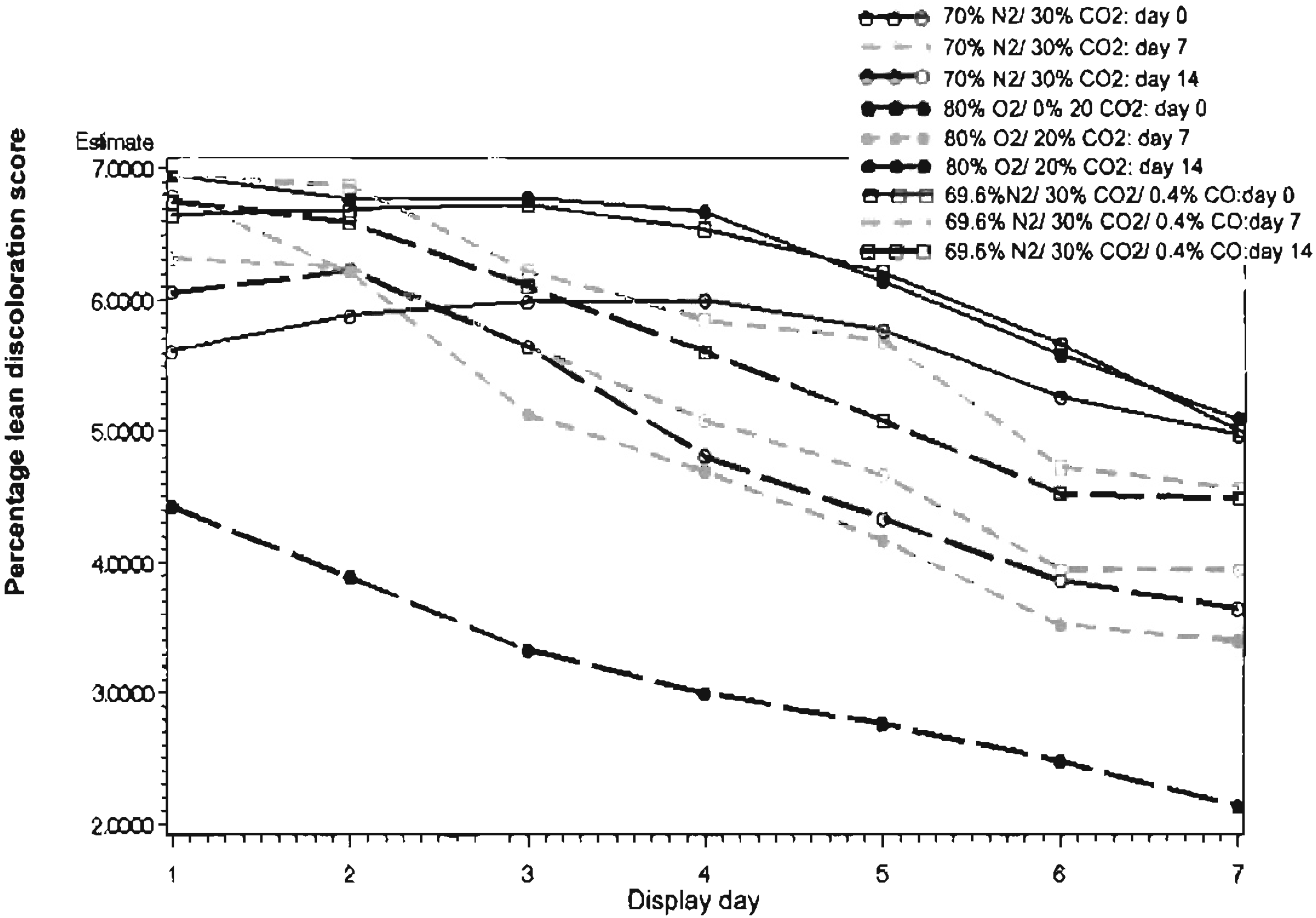
[a] Lean color: 8= bright cherry red; 4= moderately dark red or gray; 1= extremely dark gray.

Figure B. Influence of storage period, modified atmosphere composition, and display day on the lean color[a] of the *Psoas major* muscle in the T-bone steak.



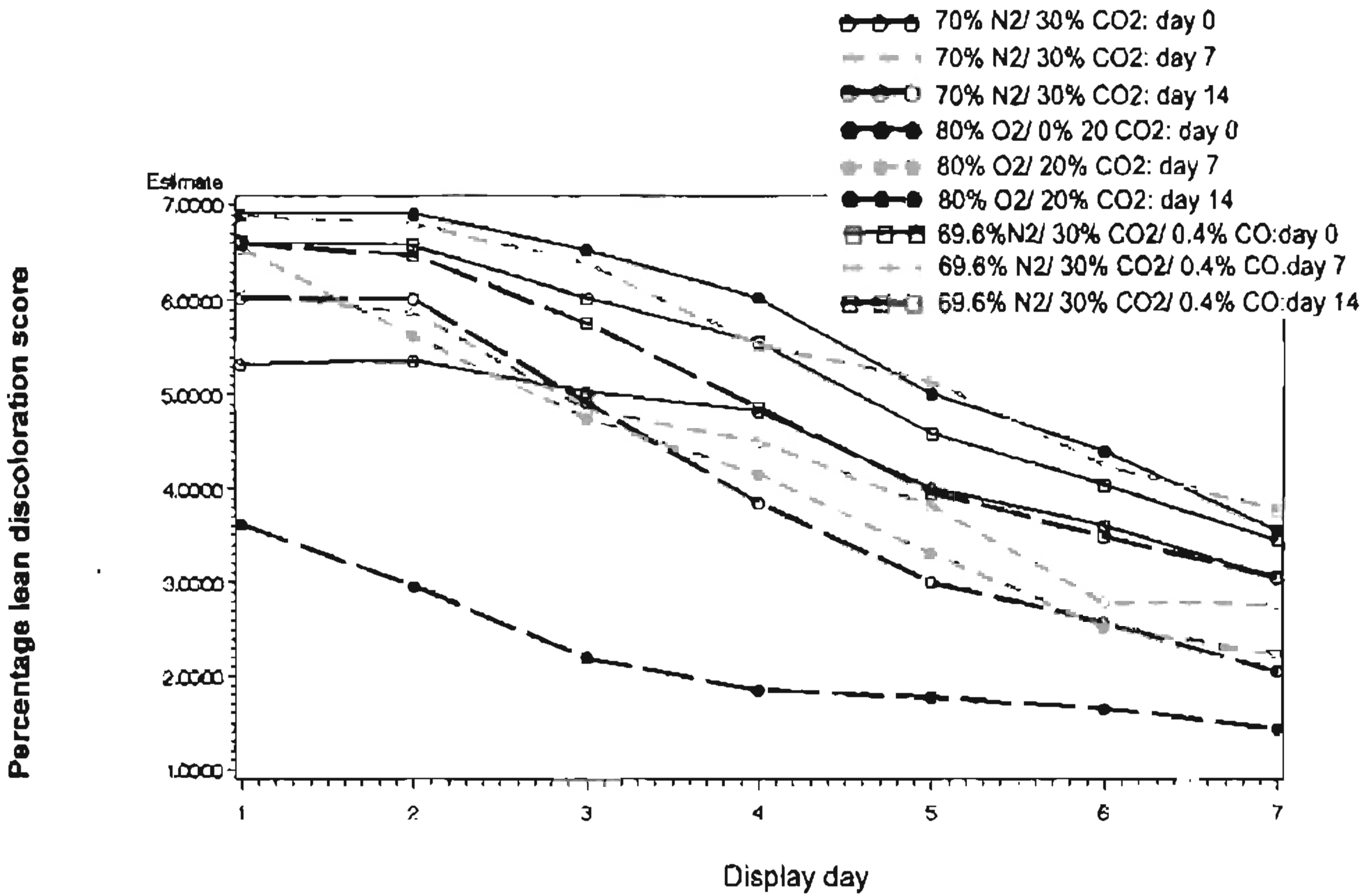
[a] Lean color: 8= bright cherry red; 4= moderately dark red or gray; 1= extremely dark gray.

Figure C. Storage time and modified atmosphere effect on percent muscle discoloration[a] for *Longissimus dorsi* muscle in T-bone steaks.



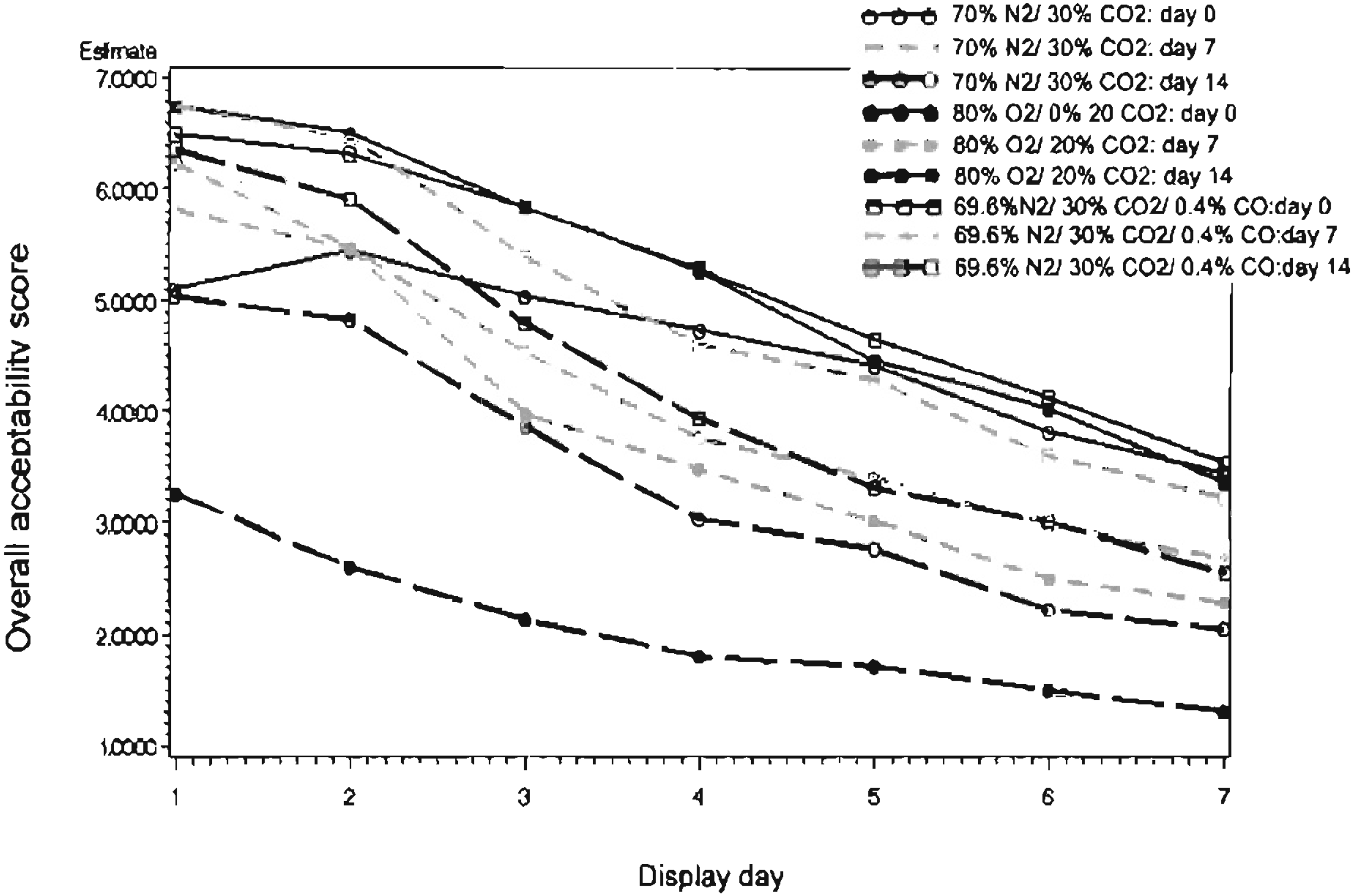
[a] Percentage discoloration: 1= 100%; 4= 26-50%; 7= 0%.

Figure D. Storage time and modified atmosphere effect on percent muscle discoloration[a] for *Psoas major* muscle in T-bone steaks.



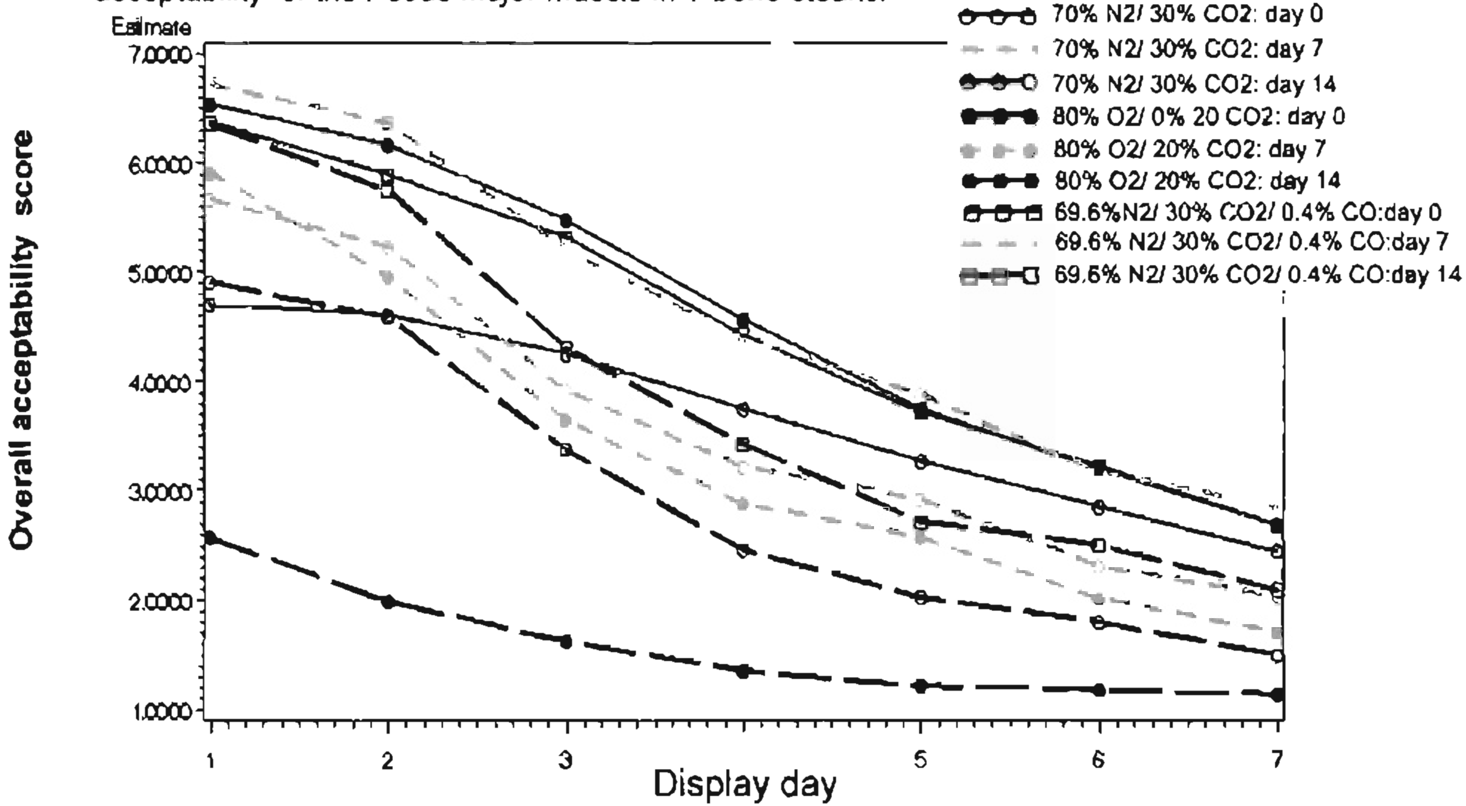
[a] Percentage discoloration: 1= 100%; 4= 26-50%; 7= 0%.

Figure E. The effect of modified atmosphere, display period, and storage day on overall acceptability[a] of the *Longissimus dorsi* muscle in T-bone steaks.



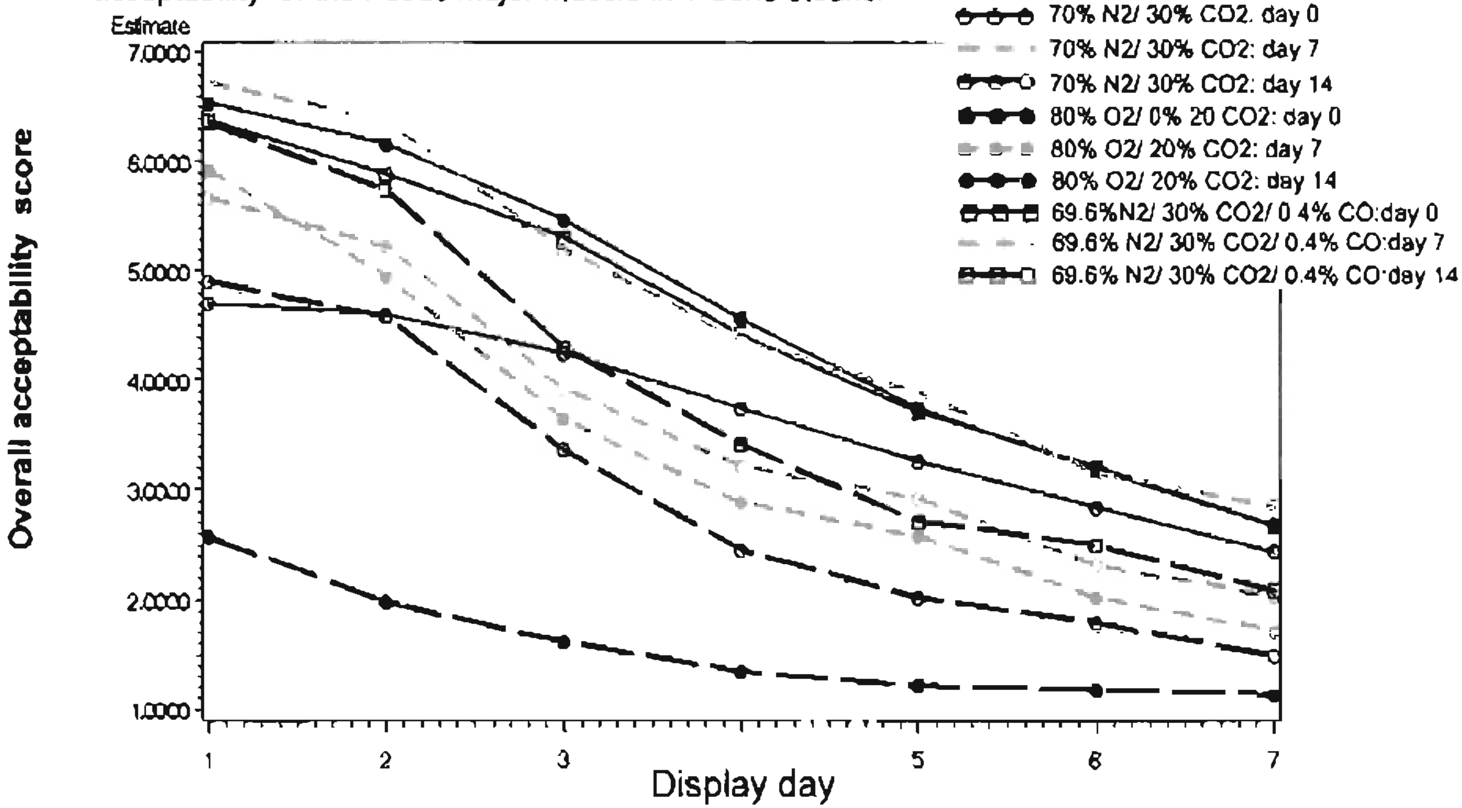
[a] Overall acceptability: 7= extremely desirable; 4= acceptable, 1= extremely undesirable.

Figure F. The effect of modified atmosphere, display period, and storage day on overall acceptability[a] of the *Psoas major* muscle in T-bone steaks.



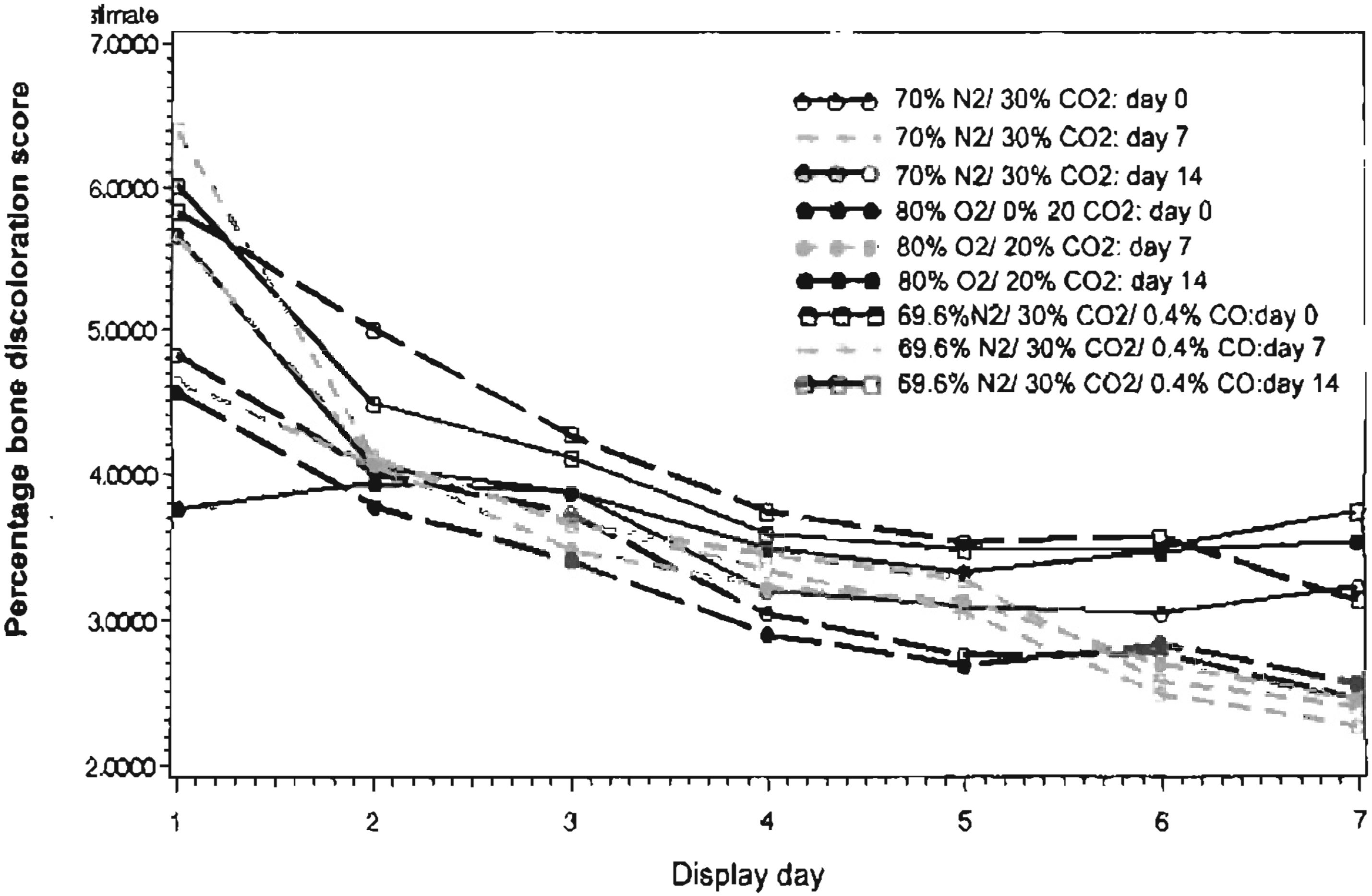
[a] Overall acceptability: 7= extremely desirable; 4= acceptable, 1= extremely undesirable.

Figure F. The effect of modified atmosphere, display period, and storage day on overall acceptability[a] of the *Psoas major* muscle in T-bone steaks.



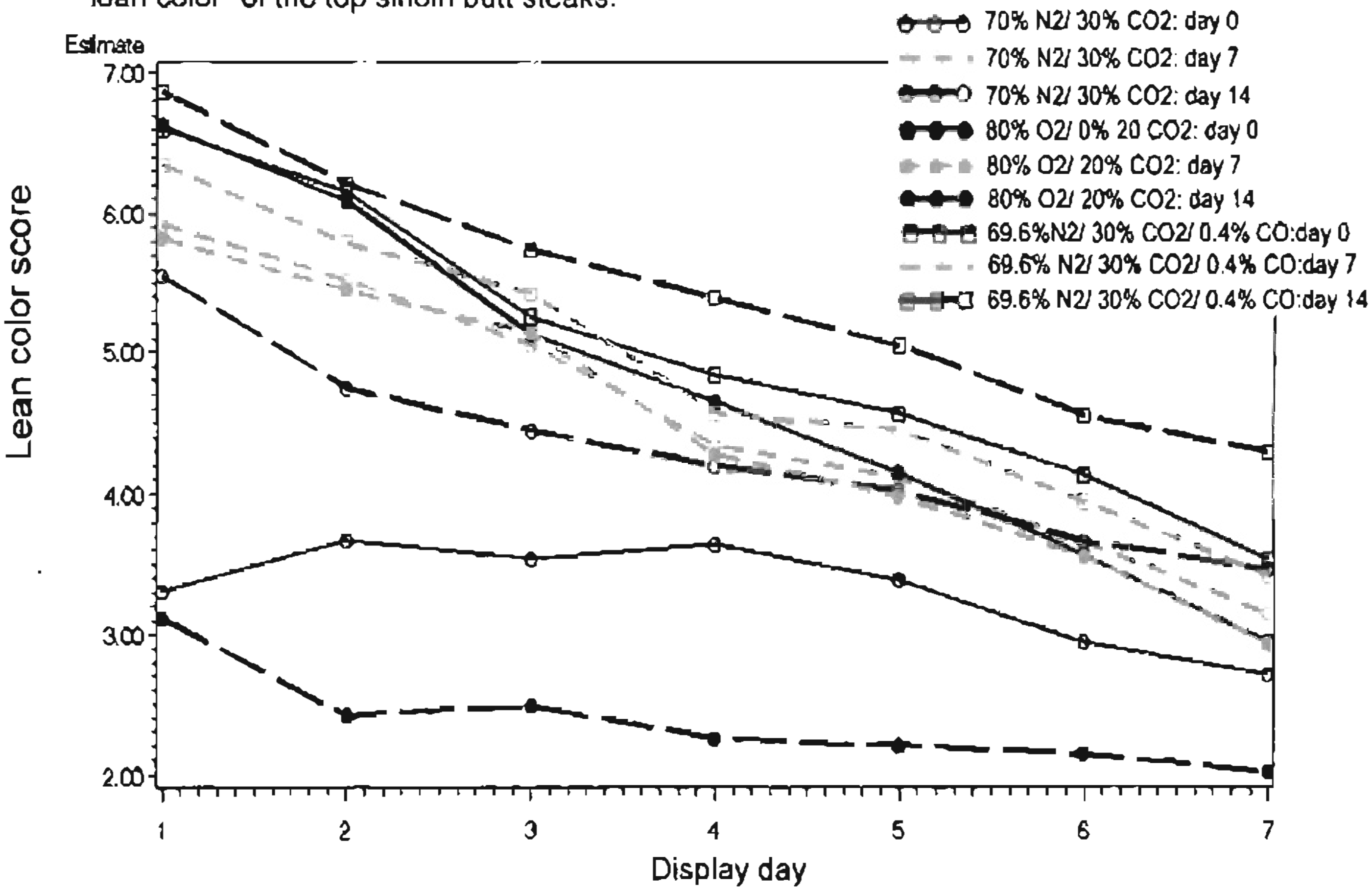
[a] Overall acceptability: 7= extremely desirable; 4= acceptable, 1= extremely undesirable.

Figure G. The effect of modified atmosphere, display period, and storage day on percent bone discoloration[a] in T-bone steaks.



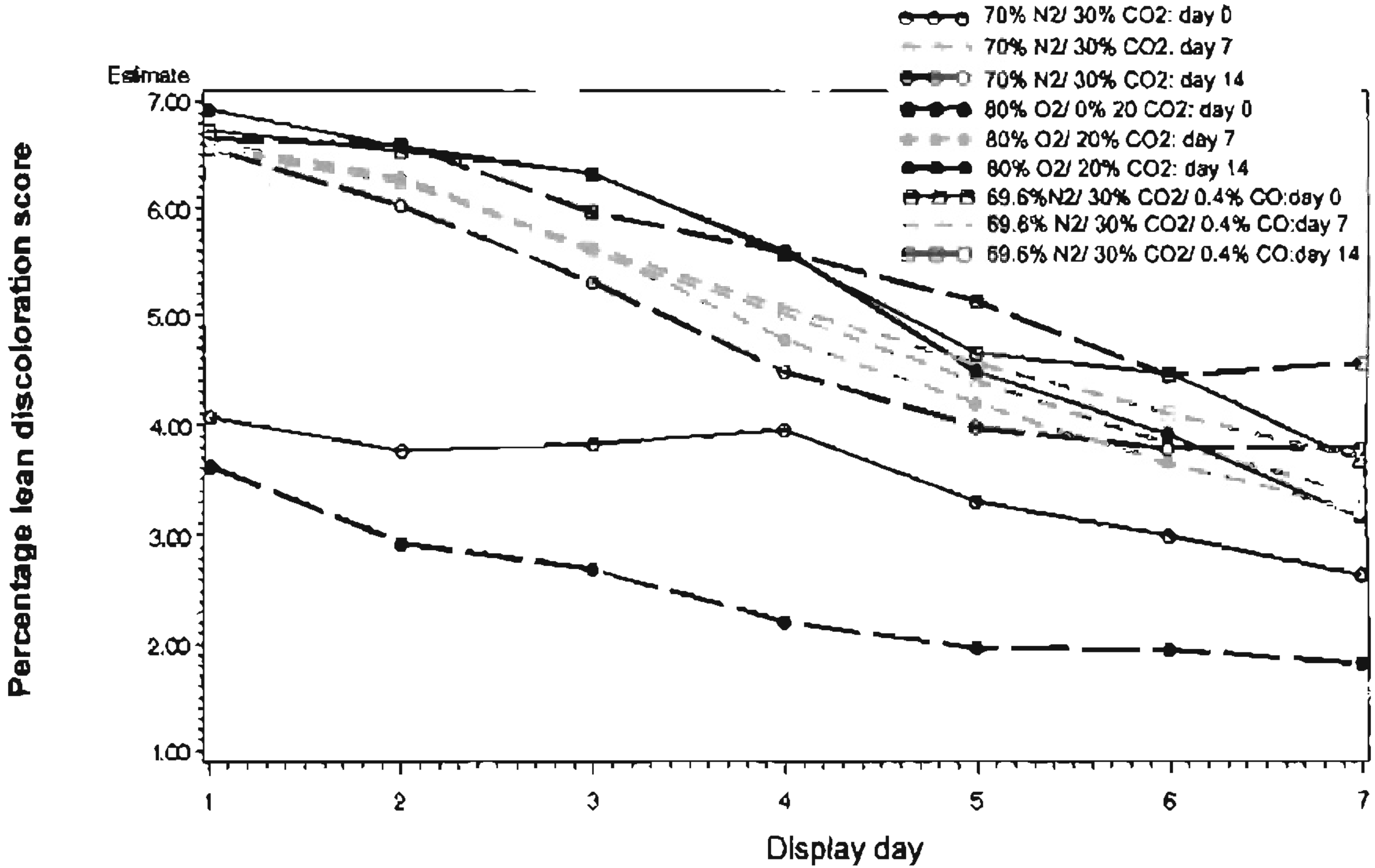
[a] Fat color: 8= creamy white; 4= slightly brown; 1= dark brown or green.

Figure H. The effect of modified atmosphere, display period, and storage day on lean color[a] of the top sirloin butt steaks.



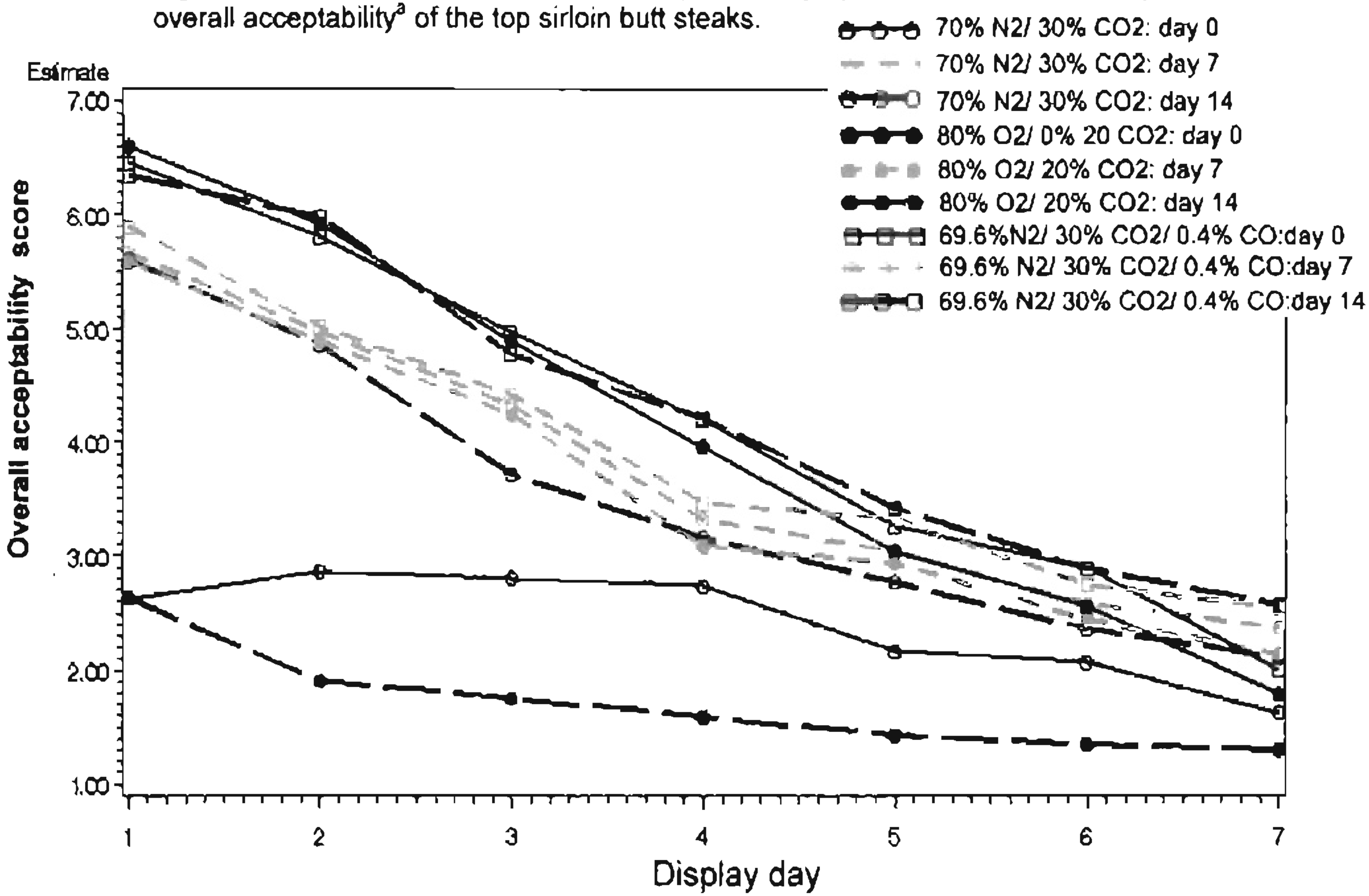
[a] Lean color: 8= bright cherry red; 4= moderately dark red or gray; 1= extremely dark gray.

Figure I. The effect of modified atmosphere, display period, and storage day on percent lean discoloration[a] of the top sirloin butt steaks.



[a] Percentage discoloration: 1= 100%; 4= 26-50%; 7= 0%.

Figure J. The influence of modified atmosphere, display period, and storage day on overall acceptability[a] of the top sirloin butt steaks.



[a] Overall acceptability: 7= extremely desirable; 4= acceptable, 1= extremelv undesirable.

CHAPTER IV

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