Use of carbon monoxide packaging for improving the shelf-life of pork

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Use of carbon monoxide packaging for improving the shelf-life of pork

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Meat Science

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Signatures have been redacted for privacy
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CHAPTER 1. GENERAL INTRODUCTION

The packaging of fresh meats has long been used as a means of preservation to extend the shelf life and the keeping quality of fresh meat. However, the shelf life of refrigerated meat is restricted by the growth of bacteria and the deterioration of color. Recent packaging applications such as vacuum and modified-atmospheres have been proven to extend the shelf life by reducing bacterial growth, but these same applications often sacrifice visual appearance for microbial inhibition. Modified atmosphere packages containing carbon dioxide, for example, have been shown to significantly increase shelf life of meat, but high levels of carbon dioxide cause a brown discoloration of the meat surface. However, another gas, carbon monoxide, may be included in the gas atmosphere to produce a stable bright-red meat color. For the past decade, Norwegian meat processors have been using a combination of the two gases, and carbon monoxide packaging has grown to a current market share of 50-60% of the Norwegian retail red meat market. Recently, Pactiv Corporation (Chicago, IL) received FDA approval on a carbon monoxide modified atmosphere packaging (MAP) system for use in the United States. The FDA notice states that carbon monoxide is generally recognized as safe (GRAS), through scientific procedures, for use as a component of a gas mixture in a MAP system. The level of CO in this MAP system is 0.4%. The other components of the MAP system are carbon dioxide (30%) and nitrogen (69.6%). However, the case-ready meats would be removed from this MAP system prior to retail display (FDA 2002). This type of atmosphere is not currently allowed in the European Union for retail packaging. Questions regarding consumer safety and shelf-life determination need to be answered before retailers will adopt this technology.
Our objectives were to investigate the potential of low levels of carbon monoxide combined with carbon dioxide in package atmospheres for extending shelf-life, improving color and reducing purge of fresh pork and injected fresh pork. We hypothesized that carbon dioxide, at high levels, will effectively inhibit microbial growth on pork chops. When combined with low levels of carbon monoxide, we theorized that the same level of microbial growth, coupled with a stable red color, would create a pork chop suitable for extended retail display and sale. The extension of shelf life is anticipated to be substantial, but a limit must be determined. Injected pork chops were included in the study to evaluate the potential for this packaging system to decrease purge loss in the individual retail packages.

**Thesis Organization**

This thesis is organized into four chapters including a general introduction, general literature review, a complete manuscript, and general conclusions. The manuscript was prepared using the Journal of Food Science Style Guide and was co-authored by Dr. Joseph G. Sebranek.
CHAPTER 2. LITERATURE REVIEW

Introduction

Meat is defined as the ‘animal tissue considered especially as food’ (Webster’s II New College Dictionary 1995). Due to its biological composition (water, protein, lipid and carbohydrate), meat is a highly perishable product and invariably goes through deterioration from slaughter until consumption (Lambert and others 1991). According to Gill (1996), two basic factors must be considered for the extension of shelf life and preservation of quality. These include the retention of an attractive appearance and the retardation of bacterial spoilage.

Color of meat is very important at the point of purchase and maintenance of this color is key for an extended shelf life. A consumer’s negative or positive reaction to color often dictates whether a cut of meat will be purchased (Lawrie 1966).

Growth of spoilage and pathogenic bacteria is of great concern to the red meat industry. The initial microbial count on meat originates from the hides of animals during slaughter and the subsequent fabrication into primal cuts (Gill and Newton 1978, Lambert and others 1991). Generally, these bacteria can be reduced in number by low temperatures and proper sanitary conditions (Sorheim and others 2001a). Advancements in technology have presented consumers with new preservation methods, and food with a longer shelf life. However, consumers are now even more aware of off-odors, off-flavors, and discolorations, which are reliable indicators of spoilage in meat (Lambert and others 1991).
Fresh Meat Color

Fresh meat color is dependent on two main factors – myoglobin concentration and the state of the myoglobin molecule (Millar and others 1996). This pigment is commonly found in three forms: deoxymyoglobin, oxymyoglobin and metmyoglobin. Deoxymyoglobin is the reduced form of myoglobin in the absence of oxygen. Deoxymyoglobin is purple-red and is often associated with vacuum-packaged products. Oxymyoglobin is the oxygenated form of myoglobin and results in a bright red pigment in fresh meat. This characteristic ‘blooming’ not only is what the consumer wants, but also is expected at the retail meat shelf. Metmyoglobin is the oxidized form of myoglobin that imparts a dull, brown color to fresh meat products. This pigment is commonly responsible for consumer rejection due to its association with aged meat (Young and others 1988).

The myoglobin pigments also serve as indicators of physical, chemical, and bacterial contaminations due to the brown color they impart with age and/or spoilage. Other factors affecting fresh meat color can be divided into intrinsic and extrinsic factors, both of which contribute to the oxidative stability of myoglobin (Fox 1987). Intrinsic factors consist of muscle pH, muscle metabolic rate, species, and age. Extrinsic factors affecting fresh meat color include temperature, oxygen availability, lighting, and microbial growth (Fox 1987).

Changes in fresh meat color are commonly measured by the use of Hunter L*a*b* values. L* values measure the lightness of an object with a value of 0 equal to black and a value of 100 equal to perfect white. The a* value is the most common value used for meat products as it is a primary indicator of redness. A positive a* value is related to redness, whereas a negative a* value represents greenness. The b* value is a measure of the degree of
yellowness represented by a positive value and the degree of blueness which is represented by a negative value (Hunt and others 1991).

**Microbiology of Fresh Meat**

Microbial growth is easily the most important factor in the keeping quality of fresh meat. Fresh meat at room temperatures has a shelf life of 1 day or less (Lambert and others 1991). At chilled temperatures, the average retail shelf life is 10-14 days (Huffman 1974). With general tendencies toward centralized retail packaging and a wider trading of retail meat, a longer storage life is needed. Centralized preparation can be more economical and even allow a more rapid distribution chain (Gill and Jones 1996). However, this may mean more time spent in transit and exposure to varying temperatures for these packaged products.

Inhibiting the bacterial spoilage of fresh meat requires a wide range of techniques. Attention must be paid to the initial load of spoilage bacteria and the temperatures at which the product is stored. Thus, initial hygienic condition of meat products is a major concern for the meat packing industry. Initial flora of meat contains both mesophilic and cold-tolerant bacteria however, only the cold-tolerant bacteria will grow at chilled temperatures. These bacteria can be separated into psychrophiles and psychrotrophs. Psychrophiles generally only occur in permanently cold environments, so spoilage floras of domestic red meat usually contain only the psychrotrophic bacteria (Gill and Newton 1980).

The primary sources of microbial contamination in meat come from the skin of animals, fecal material, or contaminates during manufacturing (Lambert and others 1991). Further cross-contamination of psychrotrophic bacteria occurs during fabrication and processing in cold environments (Young and others 1988). Improving carcass hygiene
during slaughter and processing will help achieve initial low numbers of spoilage bacteria on
fresh meat cuts (Gill 1996). Low initial microbial counts will not grow as rapidly to spoilage
numbers, thus, shelf life of a retail meat product will be extended.

A bacterial growth curve is represented in Figure 1 below. The shelf life of meat is
extended if growth can be held in the lag phase. During this phase, generation time, or time
needed for a bacterial population to double, is slow and growth is held to a minimum. The lag
phase is the initial phase of bacterial growth where the population is establishing itself in its
new environment. Once bacteria reach the exponential (log) phase, cell numbers increase
drastically and spoilage occurs at a much faster rate. Therefore, the longer bacterial growth
is held in the lag phase, the longer a shelf life extension can be achieved (Hermansen 1983).

![Figure 1. Bacterial Growth Curve.](image)

Temperature is the most important environmental factor affecting the growth of
bacteria on meat and the most important method of preservation to the meat industry (Ayres
for meat is \(-1.5 \pm 0.5^\circ C\). At temperatures below this point, meat freezes. Chill temperatures increase shelf life and product quality. The storage life of meat at 0, 2 or 5°C is about 70, 50, or 30%, respectively, of that obtained at optimum temperature (Gill 1996). Maintenance of product temperature close to optimum is important throughout the entire chill chain. Proper control of temperature needs to be recognized from production to distribution because variations in temperature allow bacteria to decrease generation time and enter logarithmic growth quickly. Despite excellent hygienic conditions and low storage temperatures, the surface of meat becomes contaminated with a variety of microorganisms.

**The Organisms**

There have been many species of psychrotrophic bacteria described, but only a few comprise the major spoilage flora of meat. Aerobic microorganisms require presence of oxygen for growth. Some of the most common Gram-negative, aerobic spoilage bacteria of meat are strains of *Pseudomonas, Moraxella, Acinetobacter*, and *Aeromonas* (Gill and Newton, 1978). Gram-positive bacteria such as *Lactobacillus* and *Brochothrix thermosphacta* are also present in high numbers on fresh meat (Dainty and others 1983).

Pathogenic bacteria of public concern have also been found in fresh beef and pork. Strains of *Salmonella, Staphylococcus aureus, Yersinia enterocolitica, Clostridium botulinum, Clostridium perfringens, Campylobacter, Aeromonas hydrophilia*, and *Listeria monocytogenes* have been isolated from fresh meat samples (Lambert and others 1991). Palumbo (1986) stated that although these bacteria remain dormant at normal refrigerated storage conditions (0-4°C), growth would occur if meat is subjected to temperature abuse and
consumer health may be threatened. The suggestion is that the growth of these potentially pathogenic organisms is inhibited when stored at low temperatures.

**Aerobic Growth**

There have been several studies conducted on the spoilage flora of meat during aerobic storage. Ayres (1960) studied the growth of bacteria on sliced beef packaged in a gas-permeable film. This study showed that fluorescent strains of *Pseudomonas* were the most prevalent in the spoilage flora. In another study conducted by Stringer (1969) on the microbial population of beef carcasses stored at chill temperatures, species of *Pseudomonas* accounted for 91% of the spoilage population. The remainder of the population was comprised of *Moraxella* and *Acinetobacter* strains. Research performed by Dainty and others (1983) showed that *Pseudomonas* strains also were the most common organisms found on beef, pork, and lamb stored in gas-permeable films (42-60% of population).

Under aerobic conditions, strains of *Pseudomonas* are the dominant spoilage organisms found in fresh meat. These microorganisms have very versatile nutritional pathways, but when available, preferentially use glucose as a substrate for growth. Since glucose is relatively abundant in meat tissue, pseudomonads can easily grow to high numbers (10^8/cm^2) before the glucose substrate becomes limiting at the muscle surface (Gill 1996). Once glucose at the surface becomes depleted, pseudomonads start to use amino acids as their growth substrate. The by-products of this amino acid breakdown produces a variety of sulfides, esters, and acids, which are identified as putrid odors and flavors (Gill 1996, Lambert and others 1991). When bacterial numbers grow to these high numbers, by-products are formed rapidly and onset of spoilage occurs shortly thereafter.
According to Gill and Newton (1977), *Pseudomonas* species have a distinct advantage in growth rate when compared to other genera on aerobically stored meat and this advantage also appears to increase with decreasing temperature. The advantage appears to be due to the inhibition of other species by *Pseudomonas*, because of their failure to compete with *Pseudomonas* for available oxygen. Once *Pseudomonas* reaches maximum cell density, it reduces both the growth rate and maximum cell densities of the other competing species (Gill and Newton 1978).

**Anaerobic Growth**

Anaerobic storage refers to the absence of oxygen during packaging, e.g. in vacuum-packaged meat. Due to the lack of oxygen in these anaerobic environments, the rapid growing pseudomonads are inhibited and bacteria such as *Brocothrix thermosphacta* and psychrotrophic enterobacteria are allowed to grow. However, in this environment, species of *Lactobacillus* dominate the micro flora at chill temperatures (Lambert and others 1991). The spoilage flora of vacuum-packaged meat will shift from the conventional micro flora to one with a greatly increased number of lactic acid bacteria present (Enfors 1979). Gill and Newton (1978) noted that *Lactobacillus* has a lower affinity for glucose compared to other competing species and can grow faster at lower temperatures than competing species as well. When maximum numbers of *Lactobacillus* are reached, these bacteria actually produce an antimicrobial agent (Roth and Clark 1975). The characteristics of this inhibitory substance have not been clearly identified, but workers have suggested that the bacteria produce an antibiotic or bacteriocin that inhibits growth of the other competing organisms in the spoilage
flora (Ahn and Stiles 1990, Gill 1996, Gill and Newton 1978). However, the role and mechanism of this bacteriocin requires more study and research.

While the dominance of lactic acid bacteria in anaerobically packaged meats greatly extends the shelf life, the meat will eventually spoil from the by-products formed by these bacteria (Ahn and Stiles 1990). Jeremiah and Gibson (1995) noted a steady increase in sourness of lactic acid spoiled pork with rejection of flavor after 5 weeks of refrigerated storage. Though lactic acid bacteria spoil meat more slowly than aerobic flora, they will still clearly cause ultimate meat spoilage when maximum population densities ($10^7$ CFU/cm$^2$) are reached (Greer and others 1993). Since the relative growth rates of meat spoilage bacteria are greatly affected by storage environment, it is important to not only delay growth of all bacteria, but to also change the storage atmosphere to prevent the growth of flora with the highest spoilage potentials (Lambert and others 1991).

**Meat Packaging**

Packaging does not improve the quality of fresh meat; it merely delays the onset of spoilage. However, the package environment can greatly affect the microbial spoilage and color life, which directly relates to an extension of shelf life. Generally, meat sold at the retail shelf is packaged in one of three ways: an oxygen-permeable overwrap, an oxygen-impermeable vacuum package, or a modified-atmosphere package. All three packaging types protect the product from outside contamination and evaporation, but the extension of shelf life is extremely different for each type (Hermansen 1983).

Large shares of fresh retail meat cuts are sold in aerobically overwrapped packages. Under these aerobic conditions, the characteristic blooming occurs and the consumer
observes an attractive bright red color. However, this packaging environment results in poor color stability and allows aerobic spoilage organisms to grow rapidly, which decreases product shelf life drastically (Sorheim 2001a). In a study by Roth and Clark (1972), sliced beef was packaged in a gas-permeable film and stored at 5°C. The results of this study showed that nearly 60% of the total aerobic plate count was comprised of fluorescent pseudomonads and *B. thermosphacta*, while species of *Acinetobacter* and *Moraxella* accounted for the rest of the spoilage organisms found on the beef. Enfors (1979) reported the microbial flora on aerobically stored fresh pork samples consisted of more than 95% Pseudomonas species. These types of microbial flora have a high potential for spoilage and reducing shelf life. To delay the growth of these microorganisms and inhibit spoilage of the meat products, the oxygen concentration must be greatly reduced. This can be achieved by vacuum or modified atmosphere packaging.

Vacuum packaging is a very common method of preservation currently used by the meat industry. The time before bacteria spoil chilled meat can be significantly extended by packages that limit the oxygen available to aerobic spoilage organisms (Jeremiah and others 1995). Vacuum packaging can simply be defined as the evacuation of air from a package that is then sealed to maintain an anaerobic environment. A good vacuum should contain <1% O₂ and concentrations of around 20% CO₂ which is produced after packaging from microbial and tissue respiration (Gill and Newton 1978, Hintlian and Hotchkiss 1986, Lambert and others 1991). The respiration activity and subsequent production of carbon dioxide is responsible for the suppression of aerobic spoilage bacteria and the predominance of facultative anaerobes such as *Lactobacillus* species (Blickstad and Molin 1983, Hermansen 1983). Due to the suppression of aerobes and a flora dominated by lactics, Gill
and Harrison (1989) stated that vacuum packaging will extend the shelf life fourfold or more compared to aerobic packaging. Film permeability is also an important factor in the extension of shelf life with vacuum packaging because small amounts of oxygen will allow *Pseudomonas* species to grow. Newton and Rigg (1979) showed that the shelf life of vacuum packaged meat decreased when packaged in films of increasing oxygen permeability.

A major disadvantage of using vacuum packaging is the negative consumer reaction to the purple color of the meat (Young and others 1988). The lack of oxygen causes the pigment to remain in the deoxymyoglobin state and appear purple-red. If residual levels of oxygen are too high in the package, color slowly changes to the grayish-brown metmyoglobin form over time. Chemical oxidation from oxygen and high levels of carbon dioxide, combined with microbial decomposition of color, cause the meat pigments to change to an undesirable brown color and give the meat product an “aged” look (Hermansen 1983).

Another disadvantage of using vacuum packages is the physical strain that is placed on both the packaging material and the meat cut. Deformation of cuts caused by the pressure on the product increases the loss of exudates from the meat cut. The loss of exudates or purge from meat products is unavoidable, but these losses contribute an unattractive appearance to the product and also an economic loss in saleable weight (Hall and others 1980). Also, due to the physical strain, vacuum bags may be punctured by bone or stress, which can cause leaks in the bags (Gill 1996, Seidman and others 1979).

In spite of these disadvantages, vacuum packaging of chilled meat eliminates external contamination and significantly prolongs the shelf life.
Modified Atmosphere Packaging

Another option to package raw, chilled meat is the use of modified-atmosphere packaging (MAP). MAP has been defined by Young and others (1988) as the enclosure of food products in high gas-barrier materials, in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbial growth and retard enzymatic spoilage – with the intent of extending shelf life. This type of packaging is well established and dates back to the 1930’s when fresh beef was shipped from Australia and New Zealand packaged under carbon dioxide (Farber 1991). Today, foods packaged in modified atmospheres include raw and cooked meats, poultry, fish, fruits, vegetables, coffee, and tea. Modified-atmosphere packages may incorporate a variety of gases singly or in combination with each other. The gases generally used in MAP of fresh meat are nitrogen (N2), oxygen (O2), carbon dioxide (CO2), and carbon monoxide (CO) (Church 1994).

Nitrogen

Nitrogen is an inert, tasteless gas with low solubility in both water and lipid tissue. The main function of nitrogen in MAP is to act as an inert filler and prevent package collapse when other gases such as O2 or CO2 are used. Nitrogen influences neither the color nor the bacterial flora on the meat (Church 1994, Hermansen 1983, Huffman 1974). However, a study done by Enfors and others (1979) showed that N2 was useful in extending the shelf life of pork. They found that the onset of spoilage took twice as long for pork stored in 100% N2 compared to pork stored in air. However, these results may be due to the exclusion of air than the direct effect of nitrogen.
Oxygen

In general, oxygen will encourage the growth of aerobic spoilage bacteria such as *Pseudomonas* species and/or *B. thermosphacta* (Silliker and others 1977). O₂ may also inhibit the growth of strict anaerobes, although there is a wide range of sensitivity to oxygen in these organisms. One of the main functions of oxygen is to keep myoglobin in the oxygenated state, oxymyoglobin. Given that low pressures of oxygen favor the development of metmyoglobin, it has been suggested by Renerre (1999) that high concentrations of O₂ be used. High concentrations of around 80% oxygen will cause the formation of metmyoglobin to occur well below the meat surface and will not show through to be seen (Hermansen 1983). However, the presence of oxygen reduces the microbial shelf life and may promote oxidative rancidity.

Carbon Dioxide

Carbon dioxide is the most important gas used in modified atmosphere packaging of fresh meat. CO₂ is both water and lipid soluble and it has been well documented that it is the main gas responsible for the antibacterial effect seen in MAP (Church 1994, Farber 1991, Wolfe 1980). Gram-negative spoilage organisms are specifically inhibited by concentrations of CO₂ over 10%, while lactic acid bacteria remain unaffected (Silliker and Wolfe 1980). There have been several theories suggested about the mechanism by which carbon dioxide inhibits growth. Wolfe (1980) claimed that CO₂ alters the intracellular pH and has consequent effects on intracellular enzyme activities and substrate transport. Other theories suggest that CO₂ inhibits the decarboxylating enzymes by mass action effect (King and Nagel 1975) or that the dissolution in cell membranes causes ensuing expansion and
disruption of membrane function (Sears and Eisenberg 1961). Regardless of the mode of action, the overall effect of CO$_2$ is an increase in the lag phase and generation time of aerobic spoilage bacteria. Despite this end result, there are many factors that contribute to not only microbial inhibition, but also the degree of this effect.

Although carbon dioxide is effective at inhibiting aerobic spoilage bacteria, it appears to have no noticeable effects on pathogens such as $S$. aureus, Campylobacter, Listeria monocytogenes, $Y$. enterocolitica, or Salmonella species (Hintlian and Hotchkiss 1986). With regard to CO$_2$-susceptible organisms, the time of application or exposure to carbon dioxide is a very important factor influencing the bacteriostatic effect. If the bacteria are exposed to CO$_2$ before growth begins, the lag phase can be extended and thereby shelf life will be extended as well. If bacteria have already entered the logarithmic phase of growth, the effect of carbon dioxide is greatly reduced. The earlier CO$_2$ is applied to a product and the lower the initial load of bacteria, the more effective MAP will be at extending the shelf life (Gill and Tan 1980).

Another important dynamic of packaging with carbon dioxide is the concentration or percentage of CO$_2$ placed in the package. Microorganisms vary in their sensitivity to CO$_2$, with molds, most yeasts and spoilage bacteria restrained by concentrations between 5 and 50% CO$_2$. Lambert and others (1991) noted that the inhibitory effect increases linearly with CO$_2$ concentrations up to 20%, but no increased inhibitory effects were noticed with concentrations $>$20%. According to Newton and others (1977), Lactobacillus species can tolerate and grow in 100% CO$_2$. Other studies have shown 100% CO$_2$ to be more effective at extending shelf life of fresh meat than concentrations of 20% (Lambert and others 1991). Hermansen (1983) reported that the optimum concentration of carbon dioxide is 15–20%.
On the other hand, Farber (1991) noted the best possible inhibition of meat spoilage bacteria occurs at concentrations of 40-60% CO₂. There are varying results on the most advantageous concentration of CO₂, but all researchers agree that carbon dioxide is the key gas in extending shelf life of fresh meat through MAP.

An additional factor influencing carbon dioxide’s effect on bacteria is the storage temperature. CO₂ is very effective at low temperatures (0°C), but at temperatures greater than 5°C, the inhibitory effect is very limited (Hermansen 1983). This increased effectiveness is due to the fact that carbon dioxide dissolves into the aqueous phase of the product more completely at lower temperatures (Genigeorgis 1985). In a study conducted by Gill and Harrison (1989) on the storage life of chilled pork under carbon dioxide, microbial spoilage was greatly delayed by lower temperature. At −1.5°C, *B. thermosphacta* was totally inhibited by CO₂. However, at 3°C, *B. thermosphacta* numbers increases substantially from 0.2% initially to 9% of the microbial flora after three weeks. Another incentive for stressing low temperatures is that a lack of refrigeration at any time during storage could allow pathogens, which are not susceptible to CO₂, to grow and possibly cause food-borne illness (Hintlian and Hotchkiss 1986).

Despite the fact that carbon dioxide is an effective bacteriostatic agent in modified-atmosphere packages, color stability in the presence of CO₂ is quite different. At elevated levels of CO₂, color darkening of the meat surface occurs due to metmyoglobin formation (Gee and Brown 1978, Kropf 1980). Previous research also indicates that concentrations of 20-30% CO₂ will discolor the meat surface. Conversely, other reports determined that 50-80% residual carbon dioxide is found in vacuum packages with no negative effect on the
meat color (Seideman and others 1980). To combat this deterioration in color, mixtures of CO₂, N₂, or O₂ have been used.

Modified atmospheres of O₂ and CO₂ are frequently used in retail packages (Gill and Jones 1996). Several studies have been done on the combination of these gases. Research by Ordonez and Ledward (1977) showed that the formation of metmyoglobin in pork muscles stored in oxygen- and carbon dioxide-enriched atmospheres at 1°C was independent of CO₂ concentration. The workers noted that increased levels of oxygen caused a significant decrease in the rate of metmyoglobin formation. A study by Silliker and others (1977) exposed beef round steak to combinations of 5-30% CO₂ and 25-65% O₂. The results showed that color after storage was improved by increasing oxygen levels and was best with 10% CO₂ and 65% O₂. They also noted that less than 50% oxygen produced brown discoloration over storage. Results have also shown that increasing oxygen concentrations results in a thicker layer of oxymyoglobin, which masks the formation of metmyoglobin by CO₂ (Kropf 1980).

Other mixtures, such as CO₂ and N₂, have been observed to extend fresh meat shelf life as well. In research done by Seidman and others (1980), beef longissimus was packaged in atmospheres of 20% CO₂/80% N₂ and 40%CO₂/60%N₂. The results showed that the 20/80 packages showed more brown discoloration than vacuum packaged cuts of beef, but the 40/60 gas mix equaled the appearance of the vacuum packages after five days of display. In 1980, Hall and others stored pork loins in 20/80, 40/60 CO₂ to N₂, and vacuum packages. They found that all three packaging environments were comparable in discoloration and odor after storage up to 28 days.
The problem with packaging in carbon dioxide is the discoloration of the meat surface, and with the inclusion of oxygen, shelf life extension is minimal. Consequently, carbon monoxide gas has been proposed to be included in MAP atmospheres so that high levels of carbon dioxide can be used and the full potential shelf life extension can be achieved.

**Carbon Monoxide**

Carbon monoxide (CO) has been recommended by many researchers to be used in modified atmospheres to improve color stability (El-Badawi and others 1964, Clark and others 1976, Sorheim and others 2001a). To induce color formation in meat, only low concentrations (<1.0%) of CO need to be included in modified-atmosphere packages. Carbon monoxide is a tasteless, colorless and odorless gas that is produced by the incomplete combustion of carbon containing materials. Carbon monoxide binds strongly to the myoglobin pigment and forms a stable cherry red color called carboxymyoglobin. The reflectance spectrum of this color is very similar to that of oxymyoglobin and imparts the "fresh" appearance to the meat surface that consumers prefer (Sorheim 1997). Also, since CO has a stronger association to the iron-porphyrin site on the myoglobin molecule, carboxymyoglobin is much more stable to oxidation than oxymyoglobin (Wolfe 1980). Additionally, data by Lanier and others (1978) has shown that the amount of metmyoglobin decreased on beef samples with CO concentrations of 1-5%. Figure 2 demonstrates the various chemical states of myoglobin. From the deoxymyoglobin state, an addition of oxygen causes the formation of oxymyoglobin and the addition of carbon monoxide causes the formation of stable carboxymyoglobin. Also, from the dexoy- or oxy- state,
metmyoglobin is formed from an oxidation reaction. This chemical state can be reversed back to deoxymyoglobin by a reduction reaction.

![Diagram showing the chemical states of myoglobin]

**Figure 2.** Various chemical states of myoglobin

Generally, the main purpose for including carbon monoxide in gas atmospheres is to induce stable color formation. However, CO may influence microbial growth as well. In a study by Gee and Brown (1980), it was found that atmospheres containing carbon monoxide have a selective action on the types of organisms that will grow in a meat culture. Growth rate studies were done with pure cultures under atmospheres of 5-30% CO. Results showed that CO had no effect on the growth of *Pseudomonas aeruginosa*, directly inhibited the growth rate of *E. coli* with increasing CO concentrations, extended the lag phase of *Achromobacter*, and prolonged both the lag phase and generation time of *Pseudomonas fluorescens*. However, the researchers stated that the use of CO at low levels (<1.0%) would probably have little effect on the microbial growth on meats. In another study conducted by
Brewer and others (1994), aerobic plate counts and lactic acid bacteria counts of beef steaks pre-treated for 30 minutes with 100% CO and vacuum-packaged were measured. Both counts were 1 log cycle lower than vacuum packaged steaks after eight weeks of storage. Additionally, Clark and others (1976) found that by adding 0.5-10% CO into N\textsubscript{2} atmospheres, the odor shelf life was extended and the growth of bacteria was reduced at 0, 5, and 10°C. Despite these anti-microbial effects, the use of low levels of CO (<1.0%) will likely be overshadowed by the anti-microbial effect of high levels of CO\textsubscript{2} (Sorheim and others 2001a).

A number of reports have been published on the packaging and treatment of fresh meat with atmospheres containing CO. Most of the studies have focused on a mixture with CO\textsubscript{2} to combine favorable color formation with an extension of bacterial shelf life. El-Badawi and others (1964) combined 2% CO with 98% air and stabilized beef color for 15 days compared to only five days in air. Conversely, Renerre and Labadie (1993) found that a CO concentration of 2% was described as “too artificial” by a sensory panel. Thus, concentrations of 0.4-1.0% CO are regarded as sufficient for color formation and stability in MAP use on meat. Luno and others (1998, 2000) combined 0.1-1.0% CO with 24 or 70% oxygen to achieve a more natural beef color. They found that at least 5.0% CO was needed to stabilize color for an extended time. In these studies, 0.1 and 0.25% CO improved color, but only at early stages of storage. A study of ground beef, pork chops and beef loin steaks packaged in 0.4% CO/ 60% CO\textsubscript{2}/ 40% N\textsubscript{2} or an atmosphere containing 70% O\textsubscript{2}/ 30% CO\textsubscript{2} was conducted by Sorheim and others (1999). The meat was stored in the dark at 4°C or 8°C for up to 21 days. Results showed that meat packaged in the CO-environment and stored at 4°C had a cherry-red color and storage lives (as indicated by off-odors) of 11, 14 and 21 days
for ground beef, beef loin steaks and pork chops, respectively. Also, meat packaged in the oxygen-containing environment resulted in initially bright red color, but the development of off-odors occurred quickly and the color was shown to be unstable.

Another way in which carbon monoxide is utilized is by pretreating fresh meat with CO and maintaining the stable red color in vacuum packages. Jayasingh and others (2001) showed extended color stability (21 days) in vacuum packages when fresh beef was pretreated with 5% CO for 24 hours or 100% CO for 1 hour. Clark and others (1976) found that pretreatment in 99% CO for 2 hours and then storage under aerobic conditions did not improve color stability. For that reason, continued storage under anaerobic conditions or in the presence of CO is crucial to preserve the bright red color of carboxymyoglobin.

Another point of concern with the stable carboxymyoglobin pigment is the internal color of the meat after it is cooked. Consumers consider internal color an excellent indicator of doneness. To address this, Sorheim (2001b) cooked beef patties that had been packaged in vacuum bags or 0.4% CO/ 60% CO2/ 40% N2. The bags were stored for 4 days at 3 °C and cooked to varying end-point temperatures of 72, 77 and 83 °C. The results showed that the carboxymyoglobin patties still had traces of pink core color immediately after slicing at all three temperatures. However, the pink color reportedly faded rapidly after exposure to air.

Despite the obvious color advantages and slight microbial inhibition by carbon monoxide, questions have been raised about the toxicity of this gas and its effect on CO concentration in human blood. CO binds to the iron atom of hemoglobin and forms carboxyhemoglobin (COHb). The affinity of hemoglobin for carbon monoxide is approximately 240 times higher than its affinity for oxygen. This binding is reversible, with a half-life of approximately 4.5 hours in persons who are at rest (Sorheim 2001a). Carbon
monoxide acts primarily in the cardiovascular system by interfering with oxygen transport, but it also can reduce the distribution of oxygen to other body tissues (Sorheim 1997).

Natural background levels of CO are quite low (0.01-0.09 mg/m³) with urban areas containing levels around 20 mg/m³. The carboxyhemoglobin concentration in the blood (COHb %) is a combination of carbon monoxide concentration in air, exposure time, and physical activity of individual (Coburn and others 1965). At COHb concentrations around 2.5%, the most sensitive individuals report chest pain. In healthy adults, no abnormal effects are described until COHb concentrations are at or near 5%. The average COHb % for non-smokers is 1.2-1.5%, and approximately 3-4% in smokers (Sorheim 2001a). Since CO has a 4.5-hour half-life in humans, short periods of elevated CO levels will significantly increase COHb levels.

The treatment of meat with carbon monoxide appears to have very little affect of COHb levels. According to Sorheim and others (1997), beef exposed to 1.0% CO for three days and cooked at 195 °C, only contained 0.1 mg of CO/kg of meat. This amounted to an 85% loss of CO in the meat. Consequently, it is highly improbable that consumption of meat packaged in a CO-MAP would raise COHb levels even to a measurable level (Sorheim and others 2001a).

Another area of concern for using carbon monoxide in MAP is worker safety. If high concentrations of CO were used and mixed with other gases at meat plants, a clear risk would be evident. The use of pure carbon monoxide in a plant setting would endanger workers from an exposure standpoint. If high levels are present, CO inhalation could be damaging (Sorheim and others 1997). The same can be said for using high concentrations of oxygen in modified atmosphere packages, as it is an explosive gas as well. By using low
concentrations of carbon monoxide, workers safety is not a major issue. This is the practice of the Norwegian meat industry. Approximately 50-60% of the retail red meat market is packaged in a low CO/high CO₂ environment (Sorheim and others 1997, 1999, 2001).

Currently, the use of carbon monoxide in the MAP of meat is not permitted in the European Union. In the United States, Pactiv Corporation is the only company with a CO system permitted for use. The Pactiv system however, does not allow meat to be packaged in this system during retail display. Therefore, full shelf-life extension may not be seen. The main features of low CO in the MAP of meat is:

- No toxic threat to consumer
- Safety in meat plant environments
- Stable, bright red color
- Long microbial shelf life; if combined with high CO₂ and removal of O₂
- Wider distribution range
- High quality product (Farber 1991, Sorheim and others 2001)

In spite of these advantages, possible negative aspects may also arise. There is a concern about the misrepresentation of a meat cut by CO packaging. The stable, red color may mask the microbial condition of the meat cut and cause consumers to misjudge the quality of a product. This potential problem is the reasoning behind Pactiv Corporation removing meat from CO packaging during retail display. Off-odors can still be detected in CO packaging however, but an end-point on shelf life must be established (Kropf 1980, Sorheim and others 1997). Other disadvantages pointed out by Hermansen (1983) include more space required during distribution and storage for MAP. In addition, these packages are not suitable for freezing.
Lipid Oxidation

Physical and chemical changes in muscle tissues during storage and utilization may alter the quality and safety of meat products. One common change in muscle foods is lipid oxidation and the subsequent deterioration of quality, including off-odor and off-flavor development (McMillin 1996, Morrissey and others 1998). Since flavor is an essential part of a consumer's eating experience, a "stale" or "rancid" taste will lead to an undesirable reaction by the consumer. These rancid flavors reduce repeat customers and must be managed at all production steps (Morrissey and others 1998). The consumer acceptability is dependent upon the extent of oxidative rancidity that has occurred (Gray 1978).

Oxidation of fatty acids, more specifically unsaturated fatty acids, is the point where lipid oxidation occurs. Oxidation of these unsaturated fatty acids develops in three phases: initiation, propagation, and termination (Figure 3). Initiation occurs when a hydrogen atom (H) is removed from an unsaturated fatty acid (RH) by bonding with oxygen or other oxidative catalysts. This produces a free radical (R•) that is extremely reactive and begins the oxidation process. Propagation results from the free radical reacting with oxygen, forming a peroxy radical (ROO•). The propagation step is where the chain reaction is set off, and more unsaturated fatty acids are oxidized once additional free radicals are produced. Termination is the step where propagation is completed and oxygen is unavailable to bind with free radicals (Gray 1978, Morrissey and others 1998).

Initiation

\[ RH + O_2 \rightarrow R\cdot + \cdot OH \]

Propagation

\[ R\cdot + O_2 \rightarrow ROO\cdot \]
ROO• + RH → ROOH + R•

Termination

R• + R• → RR
R• + ROO• → ROOR
ROO• + ROO• → ROOR + O₂

Figure 3. Mechanism for Lipid Oxidation (Gray 1978)

Initiators, which add energy to begin the oxidation process, typically include heat, freezing, water availability, light, and some enzymes (McMillin 1996). There are also several catalysts that may be present in fresh meat to induce lipid oxidation. These catalysts lower the required energy for the initiation reaction and include metal ions, high-energy oxygen, salt, or enzymes. High temperatures may also accelerate oxygen release and, consequently increase free radical production (Kanner 1994). A study by Ordonez and Ledward (1977) stored pork chops in air and observed apparent rancidity after 6 days in refrigerated storage. The researchers noted that lipid oxidation might well be a limiting factor on the shelf life of pork stored in oxygen-containing atmospheres.

Lipid oxidation in meat systems is usually measured using the 2-thiobarbituric acid test (TBA) or a modified version of this method. This test was developed by Tarladgis and others (1960) and measures the mg of malonaldehyde per 1000 g of product in a sample. Malonaldehyde is a dicarbonyl product, which is produced during the oxidation of unsaturated fatty acids. There has been a correlation coefficient of 0.89 found between the detection of rancid flavors by sensory panel and TBA number (Tarladgis and others 1960). In a study conducted by Luno and others (2000), chilled beef steaks were stored in modified atmospheres with low concentrations of CO (0.1-1.0%) in combination with O₂ (24%), high CO₂ (50%) and N₂ (25-25.9%). The investigators demonstrated by TBA analyses that
increasing concentrations of CO led to increasing inhibition of lipid oxidation. They also noted a possible antioxidant effect of CO and a subsequent extension of meat odor shelf life.

**Injected / Marinated Fresh Pork**

The production of much leaner pigs in the past few years has raised concerns that low levels of intramuscular fat may have a detrimental effect on eating quality, particularly juiciness and tenderness (Sheard and others 1999). For this reason, non-meat ingredients are added to brines and injected into meat to improve sensory characteristics of fresh pork. Specifically water, salt (NaCl), phosphate and lactates are injected to improve texture and flavor of intact, retail pork cuts. The United States Department of Agriculture Food Safety Inspection Service (USDA FSIS) regulates the limits of these ingredients in meat products. The legal limit for phosphate and lactate is 0.5% and 4.8%, respectively, based on total product weight. There is no legal limit on salt due to its self-limiting flavor profile.

Water holding in meat is a fundamental principle of meat tenderness and juiciness. Gains or losses of water are simply due to the swelling or shrinking of myofibrils (Offer and Trinick 1983). Injection of non-meat ingredients containing combinations of salt and phosphate is a well-known process that will increase the palatability of meat (Detienne and Wicker 1999). This is because salt is responsible for causing the myofibrils to swell. Offer and Trinick (1983) suggest that the Cl ions from salt bind to protein filaments and increase the electrostatic repulsion force between them. This permits the myofibrils to expand while, at the same time, the proteins take up additional water. A final salt concentration of 4.6-5.8% gives maximum water uptake; however, a concentration around 2% is commonly used in meat products due to the flavor profile (Offer and Trinick 1983).
Another non-meat ingredient used in conjunction with salt is phosphate. Phosphate-containing solutions can improve sensory characteristics and increase shelf life of a variety of meat products (Detienne and Wicker 1999). Sheard and others (1999) proposed that polyphosphate has two effects in meat – promoting the dissociation of actomyosin and promoting the depolymerization of myosin filaments. Thus, polyphosphate-treated meat would take up and retain more added water than untreated meat. This would translate into increased tenderness due to weakened muscle structure and higher water content of cooked meat. Phosphates also increase the pH of meat, which causes beneficial effects. Raising the pH from the isoelectric point increases water binding and also reduces drip loss (Cannon and others 1993, Oreskovich and others 1992). Drip loss is an important factor to the meat industry because losses decrease saleable weight and also because excessive water loss results in a less tender meat cut (Offer and Trinick 1983). Furthermore, purge leaves an unattractive appearance in the package and will also provide a medium for microbial growth (Sorheim and others 1996).

Overall, salt and phosphate work synergistically to improve water holding capacity, tenderness, and juiciness. Detienne and Wicker (1999) injected pork loins at varying salt and phosphate combinations. Their results showed a positive salt-phosphate interaction for weight gain, purge, cook loss, and expressible moisture. The action of salt and phosphate allows for the level of one to be reduced while compensating with the level of the other (Detienne and Wicker 1999).

Lactate, used in either potassium or sodium form, is another common ingredient used in fresh meat brine marinades. Lactates have been used in the food industry for many years and are utilized in the meat industry for flavor enhancement and shelf life extension.
properties (Duxbury 1988). Sodium lactate was shown by Papadopoulos and others (1991a) to have an anti-microbial effect in injected meat. Beef top rounds were injected to contain a 3% level of sodium lactate, cooked and stored aerobically at 0°C for up to 84 days. Results showed that microbial growth was decreased by addition of sodium lactate. A 1.5 log reduction of aerobic plate count was noted after 84 days of storage. In a separate study by Papadopoulos and others (1991b), cooked, vacuum-packaged beef top rounds were injected with sodium lactate at 0, 1, 2, 3, or 4%. The beef was stored for up to 84 days at 0°C. The results demonstrated that increasing levels of sodium lactate increased cook yields. Also, sensory panelists noted decreased off-flavor with increasing lactate levels; however, panelists detected a mild throat irritation at the 4% level. The researchers additionally concluded that 1% sodium lactate increased palatability, but higher levels did not further affect palatability.

**Summary**

Modified atmosphere packaging, with the use of low levels of carbon monoxide and high carbon dioxide, is clearly a system with the potential to produce a stable, bright red meat color and inhibit microbial growth. This packaging scheme could effectively increase the refrigerated shelf life of fresh pork over aerobically packaged pork and maintain a color advantage over vacuum-packaged pork cuts. The use of injection processing has been shown to increase water-holding capacity of pork during storage, thereby decreasing purge loss and improving product appearance as well as palatability. Therefore, the objective of this study was to investigate the potential advantages of carbon monoxide in package atmospheres for fresh and injected pork cuts and to evaluate the shelf life of CO-packaged pork relative to conventional aerobic- and vacuum-packaged cuts.
References


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CHAPTER 3. USE OF CARBON MONOXIDE PACKAGING FOR IMPROVING THE SHELF-LIFE OF PORK

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Abstract

The effects of packaging atmosphere (aerobic, vacuum, MAP, or MAP-CO) on pork chops were investigated. Eighty pork loins (40 injected, 40 uninjected) of normal inherent muscle quality were used to evaluate the color, microbial growth, rancidity, purge, and sensory quality of pork chops in four different packaging environments during refrigerated storage. All treatments were evaluated 3 times/week for 5 weeks during storage at 0-2°C. Hunter a* values (for both injected and uninjected chops) were significantly (P< 0.001) higher in MAP-CO (11.25) than the aerobic (6.93), MAP (3.80), or the vacuum (2.74) packages. Sensory evaluations supported this, as color values (100 point scale) were also higher for chops in MAP-CO (85.91) than aerobic (62.47), MAP (42.42), and vacuum (44.52) packages. Rancidity (TBARS) was significantly (P< 0.001) reduced during storage by MAP-CO (0.118) as compared to the aerobic packages (0.365). However, MAP-CO did not significantly reduce microbial growth or purge loss. The results showed that carbon monoxide significantly improved color stability and sensory characteristics of pork in modified atmosphere packages during refrigerated storage.

Keywords: Carbon monoxide, MAP, color, pork chops
Introduction

The use of modified atmosphere packaging (MAP) to increase refrigerated shelf life of fresh pork over that of aerobically packaged cuts has been well established (Silliker and Wolfe 1980, Farber 1993, Sorheim and others 1996) due to the inhibitory effects of carbon dioxide on bacterial growth (Blickstad and Molin 1983). The main reasons for MAP of red meats for retail sale are to prolong the microbiological shelf life and to maintain an attractive color of the product. This packaging method can provide both an extension in keeping quality and the bright red color which consumers prefer (Luno and others 1998). Advantages to MAP over vacuum packages include high levels of bacteriostatic carbon dioxide and less purge (free water) in the package than what occurs under vacuum. A disadvantage to high carbon dioxide (over 40%) is brown discoloration of meat surfaces (Silliker and others 1977, Sebranek 1985). Therefore, MAP systems for fresh meat usually utilize 20% to 30% carbon dioxide to avoid discoloration, and the full potential shelf life is not achieved.

Consequently, another gas may be included in the gas atmosphere to produce a stable bright-red meat color. It has been clearly established that carbon monoxide results in an extremely bright red color (Sorheim and others 1997a). However, carbon monoxide has not been used for MAP because of concerns for the toxicity of the gas to workers and consumers. Norwegian meat processors have used carbon monoxide at 0.5% or less (Sorheim and others 1997a). This concentration results in stable red meat color without the risk of carbon monoxide exposure and, more importantly, allows inclusion of 60% to 70% carbon dioxide in the package without discoloration. The use of this retail meat packaging has grown to a current market share of 50-60% of the Norwegian retail red meat market (Sorheim and others 1999).
Reports suggest that very significant shelf life extension can be achieved by adding carbon monoxide to MAP (Sorheim and others 1997, 1999, Luno and others 2000). Clark and others (1976) reported a stable, red color for more than 30 days for beef packaged in MA's containing 0.5-10% CO, while control samples packaged in air discolored after 5 days of storage. Health authorities, including environmental safety experts at Iowa State University, do not consider 1% or less carbon monoxide to be a significant risk to human beings. Recently, Pactiv Corporation (Chicago, IL) received FDA approval on a carbon monoxide modified-atmosphere packaging system. The FDA notice states that carbon monoxide is generally recognized as safe (GRAS) for use as a component of a gas mixture in a MAP system. The level of CO in this MAP system is 0.4%. The other components of the MAP system are carbon dioxide (30%) and nitrogen (69.6%). However, case-ready meats are required to be removed from this MAP system prior to retail display (FDA 2002).

For these reasons, use of carbon monoxide at less than 1% in MAP should allow increased concentrations of carbon dioxide for extending the shelf life of pork. In addition, MAP systems may permit packaging of injected fresh pork with reduced purge and improved product appearance. Therefore, the objectives of this study were to investigate the potential of low levels of carbon monoxide combined with carbon dioxide in package atmospheres for extending shelf life, improving color and reducing purge of fresh pork and injected fresh pork.
Materials and Methods

The experimental design utilized eight treatments of pork chops, each of which were replicated for a total of 16 observations. The experiment used a 2 x 4 factorial arrangement, with the packaging treatments as follows:

1. Uninjected aerobic overwrap
2. Uninjected vacuum
3. Uninjected MAP
4. Uninjected MAP-CO
5. Injected aerobic overwrap
6. Injected vacuum
7. Injected MAP
8. Injected MAP-CO

Fresh, boneless pork loins were purchased from local suppliers and kept refrigerated (0°C - 2°C) until used. A total of 40 pork loins were used for each of the two replications. Loins were randomly assigned to two groups of 20 each (uninjected and injected). A Townsend Model 1450 injector (Townsend Eng., Des Moines, IA., U.S.A.) was used to inject one group of loins to a target of 112% of initial green weight using a brine containing 9.3% potassium lactate, 3.7% sodium phosphate and 2.8% sodium chloride. The sodium phosphate used was a mixture of sodium tripolyphosphate, sodium polyphosphates, and glassy sodium hexametaphosphate. CurAfos, Formula 11-2, is manufactured by Rhodia Inc., Food Ingredients (Cranbury, NJ). Next, the injected loins were vacuum tumbled with a Higashimoto Model MA_100 vacuum massager (Higashimoto Kikai Co., Ltd., 1149 Mikadani Yamazoe, Nava, Japan) continuously for one hour at 10 revolutions/minute. Brine was added to the tumbler to achieve desired 112% pump retention. Both injected and
uninjected loins were cut into 1-inch thick chops and packaged using four packaging environments. The four packaging treatments were aerobic overwrap (high oxygen-permeable film), vacuum (high-barrier film), MAP using 20% carbon dioxide and 80% nitrogen, and MAP-CO using 0.5% carbon monoxide, 70% carbon dioxide and 29.5% nitrogen. The aerobic-overwrapped chops were packaged by placing single chops on polyfoam trays and covering each tray with oxygen-permeable Resinite, RMF 61-Hy, 1400cc O₂/100in²/24h at 23°C clear stretch meat film (Borden Packaging Inc., North Andover, MA., U.S.A.), using a single-roll overwrapper, Model 600a (Heat Sealing Equipment Manufacturing Co., Cleveland, OH., U.S.A.). “Vacuum” chops were packaged under vacuum by placing single chops in high barrier pouches, Curlon Grade 861, 3cc O₂/645cm²/24h at 23°C and 0% RH (Cryovac Division W.R. Grace Co., Duncan S.C., U.S.A.), using a Multivac vacuum-packaging machine (Model 1960/10, type AG800, W. Germany). MAP and MAP-CO packaging was accomplished by placing individual chops in high-barrier pouches, Curlon Grade 861, 3cc O₂/645cm²/24h at 23°C and 0% RH (Cryovac Division W.R. Grace Co., Duncan S.C., U.S.A.), using a Multivac vacuum-packaging machine (Model 1960/10, type AG800, W. Germany) by first applying vacuum, then flushing the package with the gas mixture, applying vacuum again, flushing again and finally sealing the bag with the gas mixture contained. Gas atmospheres were achieved by purchasing cylinders of compressed gases, mixed in desired ratios. All packages were stored at 0°C - 2°C in lighted display.

Color (L*a*b*) measurements of the surface of the pork chops were made by using a HunterLab LabScan instrument (Model LS, 1500, Hunter Associated Laboratories Inc., Reston, VA., U.S.A.) using an illuminant D75 and 10° observer light source (representing
daylight @7500K) with a 1.00” port insert. Calibrations were conducted after covering the calibration plate with Saran film, to simulate retail meat packaging. Three readings were taken on random locations for each chop for each treatment. Measurements were conducted on day 1 after packaging and subsequently on days 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 29, 32, 34, and 36 after packaging.

Package purge (separated free water) was measured by first weighing an unopened package. The package was then opened, and the chops and packaging material were blotted dry with paper towels and reweighed to determine weight loss. Purge loss was calculated as a percent of the weight of chops in the packages. Measurements were conducted on day 1 after packaging and subsequently on days 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 29, 32, 34, and 36 after packaging.

Each treatment was also sampled for total plate counts and lactic acid bacteria counts to monitor microbial growth. Total plate counts were used as general indicator of bacterial spoilage. To measure the total microbial counts, the entire chop for each treatment was blended in stomacher bags (Whirl Pak, NASCO, Fort Atkinson, WI., U.S.A.) using a lab blender (Stomacher Model 400, Tekmar, Cincinnati, OH., U.S.A.). The samples were then plated onto 100 x 15mm petri plates (Fisherbrand, Fisher Scientific, Chicago, IL., U.S.A.) containing peptone diluent (DIFCO, Detroit, MI., U.S.A.) with a spiral plater (Model D, Spiral Systems, Cincinnati, OH., U.S.A.). Next, the plates were incubated for 24-48 hours at 35°C and then counted according to Vanderzand and Splittstoesser, Chapter 4 (1992).

Lactic acid counts were collected because these organisms usually dominate the microbial flora in anaerobic environments. To measure the lactic acid bacteria, the entire chop for each treatment was blended in stomacher bags (Whirl Pak, NASCO, Fort Atkinson,
WI., U.S.A.) using a lab blender (Stomacher Model 400, Tekmar, Cincinnati, OH., U.S.A.). The samples were then plated onto 100 x 15mm petri plates (Fisherbrand, Fisher Scientific, Chicago, IL., U.S.A.) containing peptone diluent (DIFCO, Detroit, MI., U.S.A.) with a spiral plater (Model D, Spiral Systems, Cincinnati, OH., U.S.A.). Next, the plates were incubated for 48 hours at 37°C and then counted according to Vanderzand and Splittstoesser, Chapter 15 (1992). Microbial measurements were conducted on day 1 after packaging and subsequently on days 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 29, 32, 34, and 36 after packaging.

Oxidative rancidity was determined during the storage period using the 2-thiobarbituric (TBA) procedure of Tarladgis and others (1960). Duplicate readings were recorded for each treatment sample. Measurements were conducted on day 1 after packaging and subsequently on days 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 29, 32, 34, and 36 after packaging.

Sensory evaluation of the chops was conducted using a twelve-member, trained panel of students, staff and faculty at Iowa State University. Evaluations of external color, appearance and odor were included in the sensory analysis. Three digit number codes were assigned randomly to each treatment sample and panelists evaluated samples using a line scale with graduations from 0-100 mm (Figure 1). The attributes measured and the parameters used as anchored descriptors were color (extreme brownish gray – extreme reddish pink), appearance (extremely undesirable – extremely desirable), and odor (no off-odor – intense off-odor). The panelists were trained in two separate sessions, prior to the first replication, to evaluate attributes that represented the extremes of each respective parameter. Color and appearance evaluations were performed separately by observing 2
Color and Odor Evaluation of Pork Loin

Panelist __________
Date __________

Please look at and smell each sample and mark your evaluation on the appropriate place on the line.

Sample # ______

Color

- Extreme brownish-gray
- Extreme reddish-pink

Appearance

- Extremely undesirable
- Extremely desirable

Odor

- No off odor
- Intense off odor

Please add any comments here:

Sample # ______

Color

- Extreme brownish-gray
- Extreme reddish-pink

Appearance

- Extremely undesirable
- Extremely desirable

Odor

- No off odor
- Intense off odor

Please add any comments here:
chops/treatment. For odor evaluation, panelists received a raw sample (20-30g) of each treatment in covered plastic containers. The panelists were then asked to open the lids and "smell" for off odors.

The experiment was conducted twice over a five-month period with a 2x4 factorial design. All data were compiled and statistically analyzed using the general linear model (GLM) procedure provided by the Statistical Analysis System (SAS Institute, Inc., 1990). The main effects were replication, treatment, and storage day. The microbial data was transformed by log transformation to account for the exponential growth rates of bacteria. The growth rates were expressed in graph form so that regression modeling of the data could be performed on the slopes, while allowing for censoring (small values below 1). Least squares means were used to determine level of significance at P<0.05.

**Results and Discussion**

The Hunterlab was used to objectively measure the CIE L* (lightness), a* (redness), and b* (yellowness) characteristics taken on three random locations for each chop. The results, shown in Table 1, indicate that MAP-packaged chops resulted in the lowest L* values for both the uninjected and injected chops, while the MAP-CO chops showed the highest L* values (P<0.001). These results are consistent with the findings of Gee and Brown (1978) who reported that ground beef patties stored in a 1% CO atmosphere showed a markedly higher L* value. The L* values generally remained steady over the entire storage period and neither the uninjected nor the injected treatments significantly (P>0.05) affected the L* values (Figures 2 and 3).
The a* values were greatly affected by each packaging atmosphere (Table 1). After day 4, the MAP-CO treatments resulted in significantly (P<0.001) higher redness (a*) values than all other treatments for the entire storage period (Figures 4 and 5). These results are in agreement with Sorheim and others (1999). These researchers reported high a* values (~11) for pork chops packaged in a low CO mixture of 0.4% CO/60% CO₂/40% N₂ for 21 days. Color development (redness) in the MAP-CO treatments of that study typically occurred within 24 hours. However, this is not reflected in figures 4 and 5 in our study because of an equipment malfunction for the first replication in this experiment. After the malfunction was discovered, chops were repackaged on day 4, after which the a* values (redness) clearly increased. The delayed color development in figures 4 and 5 does not reflect the actual rate of color development for carbon monoxide packaging. In the second replication, measurements taken on day 1 showed that a* values were at 10.07 and 10.32 for the control and injected groups, respectively. This confirms that even a low level of carbon monoxide will result in a bright red color for pork chops overnight. The overwrap (OW) treatments resulted in significantly (P<0.001) higher a* values than either the vacuum or the MAP, but at the same time were significantly (P<0.001) lower than the MAP-CO treatments throughout the entire storage period (Table 1). The overwrapped packages clearly declined in redness with time as the color losses typical of spoilage occurred (Figures 4 and 5). Both the vacuum and MAP-packaged chops maintained low a* values due to the removal of oxygen and the inclusion of carbon dioxide, respectively, over the entire storage period. This is consistent with the findings of Brewer and Wu (1994), who observed that vacuum-packaged beef steaks had significantly (P<0.05) lower a* values than CO-treated steaks over a five week storage period. In both the control and injected treatments, the MAP-CO packaged chops maintained
high a* values for the entire storage period. Color remained highly attractive even after 36 days (See photos – Appendix).

The b* (yellowness) values for each packaging treatment did not change as greatly as the L* or a* values. The overwrapped packages from both injection treatments were significantly (P<0.001) higher than all other treatments and the MAP treatments were significantly (P<0.001) lower than all other treatments (Table 1). Figures 5 and 6 also show that overwrap treatments were intermediate in value after day 5 of the storage period. This agrees with Sorheim and others (1997b), who found that b* values were significantly (p<0.05) higher in atmospheres containing oxygen for pork of both normal and PSE quality. Vacuum and MAP-CO packaging of the uninjected chops showed no significant (P>0.05) difference as evidenced by Figures 6 and 7. The b* values are not as clearly related to visual color of fresh meat as the L* and a* values.

Purge loss measurements are presented in Table 2. Injection, as expected, had a significant (P<0.001) effect on the purge loss for the vacuum, MAP and MAP-CO treatments. Although the overwrap treatments were not significantly (P>0.05) different for purge, the injected chops still had a slightly lower purge. The uninjected MAP-CO chops were significantly (P<0.001) higher for purge than the other three uninjected treatments. The results show nearly a 2% increase in purge by the MAP-CO treatment compared to vacuum. However, for the injected chops, the purge loss was much less than for the uninjected control. In this case, the MAP-CO treatment was not significantly (P<0.001) greater than the overwrapped or the vacuum-packaged samples. The reason for increased purge in the MAP systems is not clear. Research conducted by Jeremiah and others (1995) reported significant (P<0.05) differences in purge values of pork loins in modified atmosphere. The purge losses
ranged from 2 – 4.75% in vacuum packages and from 4.5 – 6.5% in 100% carbon dioxide environments. However, the researchers also concluded that there were no significant (P>0.05) differences in pH values and therefore, dismissed the magnitudes of these purge differences as small. According to Renerre and Labadie (1993), there is conflicting evidence on drip losses in MAP systems. The authors indicated that drip losses in high CO₂-atmosphere packaging may be less than or equal to that in vacuum packages. In addition, Gill (1988) observed that high CO₂ concentrations lead to a more viscous exudates than normal. Sorheim and others (2001) have reported significantly (P<0.01) increased cooking losses from ground beef patties packaged in MAP-CO, which also implies a decreased retention of moisture. It will be important to determine the cause of these purge observations for MAP-CO packaging. It should be noted that the increased purge occurred only with uninjected chops. Injected chops showed slightly larger purge values for the MAP-CO packages compared with the overwrapped chops but the difference was not statistically significant (P>0.05).

Due to differing initial microbial numbers between replications 1 and 2, the microbiological analysis showed no significantly different (P>0.05) results at any point during the storage period. Variability was increased because the loins in the second replication started with relatively high microbial numbers. The high initial numbers probably masked treatment differences in the second replication. Review of the data from the first replication suggests differential effects on microbial counts by the packaging treatments. In figure 8, for example, the number of days required to reach a 10⁶ count is 7 for overwrapped packages, 23-28 for vacuum and MAP, and 36+ for MAP-CO. This suggests greater effectiveness by the MAP-CO system when microbial counts are initially low.
Figures 8-15 show the logarithmic growth for the microbial counts and are separated into replication 1 and replication 2 to distinguish the differences in the two replications. Samples with microbial counts of less than log_{10} CFU/g of 1.0 are not plotted because small values below 1.0 are automatically deleted from the statistical analysis. The microbial data were also expressed in graph form to measure the slopes and utilize a regression model. The data was transformed to logs to account for the exponential growth rates of bacteria. The least squares means of the regressions are presented in Table 3. The more rapid growth rate in the overwrapped packages is obvious in Table 3. There is no difference among the other package treatments in this comparison.

The TBA (2-thiobarbituric acid) measurements are presented in Table 4. The results showed a significant (P<0.001) difference between uninjected overwrapped (OW), injected OW, injected MAP and the remaining five treatments. The uninjected overwrap treatment was the highest for TBA values. This was expected due to the oxygen exposure of the overwrapped product and the more rapid development of rancidity. The injected product in the overwrap package was lower probably due to the antioxidant contributions of lactate and phosphate. The overwrap treatments were eliminated from the study on day 25 due to their very obvious spoilage, indicated by color and sensory observations. Other than the overwrap treatments, there were no major differences in TBA values between the other treatments; all were equally effective for suppressing rancidity development. These results are in agreement with Luno and others (2000), who noted that increasing levels of CO over 0.25% led to increased (P<0.01) inhibition of oxidation in beef steaks over a 29 day storage period.

Sensory characteristics were measured using a 12-member trained panel. Samples were evaluated on all 16 sampling days. The characteristics measured included color
(desirable-undesirable), appearance (desirable-undesirable) and odor (no off odor-extreme off odor) each utilizing a 100 point scale. The sensory data are shown in Table 5.

Results during the first sample evaluations indicated that in both of the injection groups, the MAP-CO treatments received very significantly \((P<0.001)\) higher scores for color. The high sensory color score is supported by the high a* values discussed earlier for the MAP-CO treatments. The results also indicated that the panel scored the appearance of the MAP-CO chops and the overwrapped chops higher for both of the injection groups. The odor scores showed no significant \((P>0.05)\) differences over the entire storage period. Samples were removed from the study when color deterioration was obvious thus avoiding the exposure of panelists to extreme off-odors generated by spoilage.

The color scores for each test day over the entire storage period are shown in Figure 16 and 17. As evidenced by the figures, MAP-CO received significantly \((P<0.001)\) higher scores from the panel for both the uninjected and injected chops. Contrasting results were reported by Renerre and Labadie (1993) in a study of beef stored in a MA of 2% CO/78% CO$_2$/ 20% N$_2$. Results showed that color of the meat was characterized as "too artificial" by a sensory panel. However, research by Luno and others (1998) and Sorheim and others (1999) showed that sensory analysis of visual red color produced by CO received significantly \((P<0.05)\) higher scores. The overwrap treatment (Figure 16) decreased rapidly in color score compared to the other treatments and was not evaluated after day 25 due to obvious spoilage. MAP and vacuum treatments were recognized as the least desirable \((P<0.001)\) in terms of color, but the scores remained relatively steady for the entire storage period.
Appearance scores were more similar than color scores between the treatments according to sensory data. This probably reflects a more generalized evaluation of "appearance" by the panel as opposed to a more specific "color" characteristic. The overwrap treatment generally received higher scores for appearance than the other treatments during the first two weeks. The relatively high appearance score for overwrapped chops may reflect the panelists assessment of a traditional expected appearance (overwrapped tray similar to current retail packages) as opposed to the other packaging systems. The MAP chops earned significantly (P<0.001) lower scores, in both uninjected and injected groups, than any other treatments. Figures 18 and 19 show the appearance values over the storage period. The deterioration over time for the overwrapped chops is obvious and clearly results from the change in color noted previously.

Conclusions

The results of this study show that low levels of carbon monoxide (0.5%) in a modified atmosphere package achieved a dramatically stable, bright-red color over an extended storage period. The carbon monoxide treatment also suppressed lipid oxidation when compared to overwrap package treatment. The results, however, also indicated that modified atmosphere packages containing low carbon monoxide and high carbon dioxide increased purge loss of uninjected pork chops. The injection treatment of pork chops prevented the purge effects observed for carbon monoxide packaging on uninjected chops. These findings suggest that the shelf life of fresh pork, particularly in regard to color, can be increased with carbon monoxide packaging. However, observations of increased purge from uninjected pork chops warrant further research.
References


Brewer MS, and Wu S. 1994. Carbon monoxide effects on color and microbial counts of vacuum-packaged fresh beef steaks in refrigerated storage. J Food Quality 17:231-244.


Table 1. The effect of packaging atmosphere on the least squares means of L* (lightness), a* (redness), and b* (yellowness) values for pork chops.

<table>
<thead>
<tr>
<th>Color Measure</th>
<th>Uninjected chops</th>
<th>Injected chops</th>
<th>S.E&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
</tr>
<tr>
<td>L*</td>
<td>49.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.32&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>48.65&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>a*</td>
<td>6.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.74&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.80&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>b*</td>
<td>12.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.51&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of means.

Means within same row with different letters are significantly different at P<0.05

Packaging atmospheres:
- OW = overwrapped
- Vacuum = vacuum-packages
- MAP = modified-atmosphere of 20% CO₂, 80% N₂
- MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂
Figure 2. Least squares means for the L* values of uninjected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂ (S.E. = 1.32).
Figure 3. Least squares means for the $L^*$ values of injected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$ (S.E. = 1.32).
Figure 4. Least squares means for the $a^*$ values of uninjected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$ (S.E. = 0.23).
Figure 5. Least squares means for the $a^*$ values of injected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$ (S.E. = 0.23).
Figure 6. Least squares means for the b* values of uninjected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂ (S.E. = 0.54).
Figure 7. Least squares means for the b* values of injected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO₂, 97% CO₂, 29.5% N₂ (S.E. = 0.54).
Table 2. The effect of packaging atmosphere on the least squares means for purge values of pork chops.

<table>
<thead>
<tr>
<th></th>
<th>Uninjected chops</th>
<th></th>
<th></th>
<th></th>
<th>Injected chops</th>
<th></th>
<th></th>
<th></th>
<th>S.E\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
<td>MAP-CO</td>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
<td>MAP-CO</td>
</tr>
<tr>
<td>Purge (%)</td>
<td>1.25\textsuperscript{e}</td>
<td>2.63\textsuperscript{cd}</td>
<td>3.53\textsuperscript{bc}</td>
<td>4.53\textsuperscript{b}</td>
<td>0.94\textsuperscript{e}</td>
<td>1.19\textsuperscript{e}</td>
<td>2.18\textsuperscript{d}</td>
<td>1.46\textsuperscript{de}</td>
<td>0.24</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Standard error of means.

\textsuperscript{b,c}Means within same row with different letters are significantly different at P<0.05

Packaging atmospheres:
- OW = overwrapped
- Vacuum = vacuum-packages
- MAP = modified-atmosphere of 20% CO\textsubscript{2}, 80% N\textsubscript{2}
- MAP-CO = modified-atmosphere of 0.5% CO, 70% CO\textsubscript{2}, 29.5% N\textsubscript{2}
Table 3. Regression values for the least squares means of microbial growth (CFU/g) on pork chops as a result of packaging atmosphere.

<table>
<thead>
<tr>
<th>Regression values</th>
<th>Uninjected chops</th>
<th></th>
<th>Injected chops</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
<td>MAP-CO</td>
</tr>
<tr>
<td>Aerobic</td>
<td>.281</td>
<td>.093</td>
<td>.160</td>
<td>.099</td>
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<tr>
<td>Anaerobic</td>
<td>.321</td>
<td>.184</td>
<td>.187</td>
<td>.098</td>
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</tbody>
</table>

Means within same row are not significantly different. (P>0.05)

Packaging atmospheres:
- OW = overwrapped
- Vacuum = vacuum-packages
- MAP = modified-atmosphere of 20% CO₂, 80% N₂
- MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂
Figure 8. Replication #1 aerobic plate count values ($\log_{10}$ CFU/g) on uninjected pork chops during storage. (OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$)

Figure 9. Replication #1 aerobic plate count values ($\log_{10}$ CFU/g) on injected pork chops during storage. (OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$)
Figure 10. Replication #2 aerobic plate count values (Log$_{10}$ CFU/g) on uninjected pork chops during storage. (Overwrap = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$)

Figure 11. Replication #2 aerobic plate count values (Log$_{10}$ CFU/g) on injected pork chops during storage. (Overwrap = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$)
Figure 12. Replication #1 lactic acid bacteria plate count values (Log_{10} CFU/g) on uninjected pork chops during storage. (Overwrap = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂)

Figure 13. Replication #1 lactic acid bacteria plate count values (Log_{10} CFU/g) on injected pork chops during storage. (Overwrap = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂)
Figure 14. Replication #2 lactic acid bacteria plate count values (Log$_{10}$ CFU/g) on uninjected pork chops during storage. (Overwrap = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$)

Figure 15. Replication #2 lactic acid bacteria plate count values (Log$_{10}$ CFU/g) on injected pork chops during storage. (Overwrap = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO$_2$, 80% N$_2$, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO$_2$, 29.5% N$_2$)
Table 4. The effect of packaging atmosphere on the least squares means of TBA values for pork chops.

<table>
<thead>
<tr>
<th></th>
<th>Uninjected chops</th>
<th></th>
<th>Injected chops</th>
<th></th>
<th>S.E&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
<td>MAP-CO</td>
<td>OW</td>
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<tr>
<td>TBA Values</td>
<td>.365&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.096&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.111&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.118&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.198&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of means.

<sup>b-c</sup>Means within same row with different letters are significantly different at P<0.05

Packaging atmospheres:
- OW = overwrapped
- Vacuum = vacuum-packages
- MAP = modified-atmosphere of 20% CO<sub>2</sub>, 80% N<sub>2</sub>
- MAP-CO = modified-atmosphere of 0.5% CO, 70% CO<sub>2</sub>, 29.5% N<sub>2</sub>
Table 5. The effect of packaging atmosphere on the least squares means for sensory characteristics (100-point scale) of pork chops.

<table>
<thead>
<tr>
<th>Sensory property</th>
<th>Uninjected chops</th>
<th>Injected chops</th>
<th>S.E&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
</tr>
<tr>
<td>Color</td>
<td>62.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42.42&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Appearance</td>
<td>62.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.17&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>46.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Odor</td>
<td>35.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of means.
<sup>b-d</sup>Means within same row with different letters are significantly different at P<0.05

Packaging atmospheres:
- OW = overwrapped
- Vacuum = vacuum-packages
- MAP = modified-atmosphere of 20% CO₂, 80% N₂
- MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂
Figure 16. Least squares means for the color values of uninjected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂ (S.E. = 8.84).
Figure 17. Least squares means for the color values of injected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO₂, 70% CO₂, 29.5% N₂ (S.E. = 8.84).
Figure 18. Least squares means for the appearance values of uninjected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂ (S.E. = 7.99).
Figure 19. Least squares means for the appearance values of injected pork chops during storage. OW = overwrapped, Vacuum = vacuum-packaged, MAP = modified-atmosphere of 20% CO₂, 80% N₂, and MAP-CO = modified-atmosphere of 0.5% CO, 70% CO₂, 29.5% N₂ (S.E. = 7.99).
APPENDIX

Day 6
Day 11
Day 27
Day 36
CHAPTER 4. GENERAL CONCLUSIONS

The use of low-level carbon monoxide packaging system has the ability to extend the overall shelf life of fresh and injected fresh pork. This modified atmosphere packaging system demonstrated a stable, bright-red color over the entire storage period. Our results revealed that the carbon monoxide modified atmosphere packages provided the highest \( L^* \) and \( a^* \) values \((P<0.001)\) over the 36-day storage period when compared to the other three packaging environments (overwrap, vacuum and MAP). Since color is the most important factor affecting consumers' interpretation of fresh meat quality, we can conclude that the dramatic color retention would be beneficial on the retail meat shelf.

Carbon monoxide MAP packages did, however, have unexpected negative effects on the purge values (%) of pork chops. The uninjected chops in the MAP-CO (4.53%) treatment had significantly \((P<0.05)\) more drip loss than vacuum packaged (2.63%) chops. This difference was not seen in the injected chops. In addition, we had hypothesized that the microbial shelf life would be extended due to the inhibitory effects of carbon dioxide, but microbial analysis showed no significant \((P>0.05)\) differences in the onset time of spoilage numbers. Due to the fact that color shelf life masked the onset of spoilage, a limit on shelf life needs to be set for the MAP-CO treatment to be a success. On the other hand, TBA values of the MAP-CO chops were significantly \((P<0.05)\) suppressed when compared to the overwrapped chops. Sensory analysis of color also indicated that MAP-CO packages maintained an extremely reddish pink color over the entire storage period. Therefore, our results suggest that the overall shelf life of fresh pork can be increased with this carbon monoxide packaging system.
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