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EFFECT OF THE CO2 GRINDING ON MODIFIED ATMOSPHERE AND COLOR SHELF LIFE OF GROUND BEEF

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EFFECT OF THE CO2 GRINDING ON MODIFIED ATMOSPHERE AND COLOR SHELF LIFE OF GROUND BEEF

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ABSTRACT

Beef ground under air and carbon dioxide (CO_2) conditions were evaluated for their storage stability at 2 ± 1ºC starting after 2 days in the dark to simulate transportation, followed by 7 days in lighted display by measuring color (CIE L*, a*, b*, the hue and chroma), gas headspace, microbiological content, and pigment content. AC (ground in air then packaged in 100% CO_2 MAP) and CC (ground in CO_2 then packaged in 100% CO_2 MAP) treatments showed the highest a* and chroma values from day 3 to 9 compared with AV (ground in air then vacuum packaged) and CV (ground in CO_2 then vacuum packaged) demonstrating greater color stability. CO_2 grinding was effective in inhibiting total aerobic bacteria ($P<0.05$) and provided a 1.2 log reduction in microbial population during 9 days storage compared to meat ground in air.

Oxymyoglobin (OxyMb) content remained in an acceptable level (45- 48%) at day 9 for AC and CC ground beef packages, and surface metmyoglobin (MetMb) also remained in an acceptable range between 24 to 31% during display for all four treatments. However, ground beef in vacuum packaging (AV and CV) developed and maintained a high myoglobin (Mb) content which explains the decrease in OxyMb from 55 to 0% and 35 to 0.5% for AV and CV, respectively throughout storage.

DEDICATION

I dedicate this thesis to my wife, Imen, and to our son, Adam. I thank you for your constant support, and encouragement through the course of my master's degree. This work would have never been achieved without my wife's help and support to finally reach my goal.

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INTRODUCTION

The consumption of ground beef in the United States has steadily increased over the past decade. Meat discoloration costs the meat industry millions of dollars in lost sales in the retail market. There have been numerous attempts to prevent surface browning, which occurs rapidly in high oxygen environments, to extend the shelf-life of fresh meat products. One of the most promising treatments to slow meat discoloration involves dietary supplementation with vitamin E, which has been shown to be an effective means to extend color stability of modified atmosphere packaged (MAP) meat (Gatellier et al., 2001. The use of case-ready MAP has grown substantially. In 2004, 60% of packages audited were in case-ready format compared with 49% just two years earlier (Eilert 2005). Traditional Styrofoam TM trays with polyvinyl chloride (PVC) wrap declined from 51% in 2002 to 47% in 2004 while modified atmosphere packages experienced a 4 % growth during the same period (Eilert 2005). However, the industry continues to search for ways to increase color shelf-life of ground beef that is related to the bright red oxymyoglobin appearance which is often also associated with freshness and influences the decision for consumers to buy or reject the meat product.

Nearly 15% of retail beef is discounted in price due to surface discoloration which corresponds to annual revenue losses of $I billion (Smith et al., 2000). Renere and Labas (1987) reported that consumers begin to discriminate steak color when approximately 20% MetMb is present. Reflectance measurements of meat surfaces have been used extensively to measure the proportion of meat pigments Mb, OxyMb and

MetMb by using K/S absorption scattering values; [the ratios of absorption coefficients to scattering coefficient]. (Dean et al., 1960; Snyder, 1965; Stewart et al., 1965; Van den Oord et al., 1971; Krzywicki, 1979). The K/S ratio measured at specific reflectance wavelengths has also been used to quantitate meat pigments. Others researchers have reported that detectable changes in beef color occur after the (K/S) 572/ (K/S) 525 value has decreased by 0.16 units or to 20% MetMb concentration (O'Keefee& Hood, 1982).

MAP for red meat destined for retail sale prolongs the microbiological shelf –life and, in some cases, product color (Luno et al. 1998). Modified atmosphere gases usually include carbon dioxide, oxygen and/or nitrogen. Carbon dioxide inhibits the growth of many microorganisms, but high concentrations (over 40%) may result in brown discoloration on meat surfaces (Silliker et al.1977). Conversely, high oxygen concentration allows meat to bloom to a bright red, color, because of the formation of oxymyoglobin, but may also accelerate the growth of aerobic microorganisms. Nitrogen is neither bacteriocidal nor color stabilizing, except by creating a reduced oxygen environment and by preventing packaged from collapse because it is not absorbed as readily by meat as is O2 or CO2 (Sorheim et al. 1997).

The effectiveness of MAP on meat preservation is generally determined by the amount of CO2 dissolving into the food. However, the optimum concentration of CO2 for maximum shelf life has not been established, but in general, the literature indicates that increased amounts of CO2 extends shelf life (Hotchkiss and Langston 1995).

According to Sivertsvik et al. (2004a), a sufficient amount of CO2 can be dissolved into the product during 1 to 2 h in pure CO2 before retail packaging. This

method is called soluble gas stabilization (SGS) (Sivertsvik 2000, 2003). SGS has the potential to prevent package collapse even at high filling degree without compromising meat quality and increasing package efficiency.

Dissolving CO2 into ground meat during grinding process and before packaging is the objective of this study. The impact of using 100% CO2 during grinding and 100% CO2 or vacuum packaging on extending the storage shelf life compared to air grinding with 100% CO2 or vacuum packaging were investigated.

LITERATURE REVIEW

Pre-slaughter and post-slaughter factors affecting meat color

Slowing discoloration in retail meats during display conditions can be delayed by reducing the formation of metmyoglobin if dietary vitamin E supplementation is implemented through preharvest diet, by playing an antioxidant role in cell membranes (Linder, 1985). It was estimated that increasing the color shelf life of beef by 2 days would save $792 million annually in US retail beef sales (Liu et al., 1995). Lynch et al., (2002) reported that breed, feeding regime, and housing influenced display color variability of beef. In general, feeding effects on color were attributed to the relationship between lipid (particularly of polyunsaturated fatty acids) and pigment oxidation (Mancini et al. 2005). Further research conducted by O'Sullivan et al. (2003) reported that dietary treatment between high herbage versus conventional feeding conditions had no significant effect on the color of overwrapped longissimus steaks, whereas longissimus color stability of steaks from high-herbage diets packaged in high-oxygen MAP was improved compared to longissimus cuts from cattle fed straw and other low quality forages. This was attributed to dietary effects through accumulation of lipid-soluble antioxidants and reduced intramuscular fat. Other researchers reported that muscle type and breed had a great influence on color shelf life (O'keefe et al., 1982; Renerre, 1984, 1987a; Faustman et al., 1991) Renerre et al (1987a) and Faustman et al., (1991). Renerre et al., (1987a) observed that the gluteus medius (GM) discolored more rapidly than longissimus dorsi (LD). Faustman and Cassens, (1991) indicated that the

rate of metmyoglobin accumulation in Holstein beef was higher than in crossbred meat and suggested that differences in their intrinsic metabolisms were important factors in meat discoloration.

Housing systems may affect beef color through changes in physical activity, which could influence muscle fiber type and metabolism. Vestergaard et al. (2000) evaluated the combined effects of free range and low feeding level followed by grazing in comparison with tie-stall housing and high feeding level of young bulls on muscle fiber characteristics and meat color. Meat from animals exposed to a free range combined with a roughage-based diet had lower a* and chroma values.

Post-slaughter changes during conversion of muscle to meat are complicated and critical processes. In general, the animal undergoes varying degrees of stress before slaughter which influences rigor mortis, and these changes can play an important role in meat quality. The pale, soft and exudative (PSE) meat, and dark, firm and dry (DFD) meat conditions are influenced by post-mortem pH changes, which dramatically affect the appearance and quality of meat. A quick post-mortem pH decline can result in PSE meat which is unattractive to consumers, loses nutrients through drip and has poor water-binding ability making it unacceptable for further processing. In contrast, if post-mortem pH stays high (>6.5) the meat can become DFD, with a high water-binding capacity, but an increased rate of a rapid microbial spoilage, and an undesirable color. Bacterial contamination of carcasses is another important factor in shortening shelf life and color stability of fresh beef products. Delaying bacterial spoilage of raw meat requires proper attention to hygiene through the entirety of meat processing. HACCP and GMP systems

are important tools for quality assurance that are implemented in the meat industry to help to achieve low bacterial loads on final products. (Gill et al., 1982). Emswiler et al., (2003), suggested that contamination not removed during carcasses washing or trimming can spread easily during processing. Because grinding exposes a greater meat surface area, ground meat is very susceptible to microbial contamination, and shortened shelf life, and also is a potential source of food-borne illness (Jimenez-Villarreal et al 2003). Corrective actions can be taken at critical control points during processing such as use of an antimicrobial, hot water washing (Ellerbroek et al., 1997; Regan et al., 1996), or steam pasteurization (Pheebus et al, 1997). Also, chemical treatments on carcasses are effective, such as organic acid rinses (Bell et al., 1986), and chlorine dioxide (Emswiler et al., 1976)

Chemistry of Myoglobin Color

One of the most important attributes involved in acceptance of meats by consumers is color (Manu Tawiah. 1991). If the color of fresh meat is not protected during shelf life, metmyoglobin will form resulting in a brown color which is often equated with spoilage of fresh meat by consumers (Ernest, 1980). The pigment most responsible for meat color is the protein myoglobin. (Hunt et al., 1997). The physiological function of myoglobin in muscle is to store oxygen. The iron of the heme group has six coordination sites-four of which are bound to the protoporphyrin, one that is attached to the apoprotein and one that is available for binding various molecules. The color is principally dependant upon the redox state of the heme iron of myoglobin (Mb)

molecule bound to the 6th position. In the case of fresh meat, the degree of oxygenation of myoglobin molecules in the tissue is often an important factor.

The appearance of muscle is predominantly determined by the state of the muscle pigment, myoglobin (Mac Dougall, 1977). In the absence of oxygen, the predominant pigment is deoxymyoglobin (DeoxyMb), which has a dull, purple color. On exposure to air, the pigment is oxygenated to form oxymyoglobin (OxyMb), which imparts a bright, red color to the meat, which consumers prefer (Jeremiah, 1982). At this point, iron is in the reduced form (+2 ferrous oxidation state) in both DeoxyMb and OxyMb. Also, both Deoxy-and OxyMb can react with oxygen to form the oxidized form, metmyoglobin (MetMb), which has a dull, brown color (Hood & Riordan, 1973). MetMb is in the ferric state (Fe3+) and the MetMb can not easily bind oxygen but binds a molecule of water in the sixth coordination site. (Linvingstone et al., 1981). Although, many small compounds can bind to the sixth coordination site, producing a range of colored compounds (Dymicky et al., 1975), only three ligands are usually important in the context of meat: oxygen (O2), nitric oxide (NO), and carbon monoxide (CO). In meat curing, several ingredients are used such as: nitrite, salt, ascorbate, sugar and spice (Casens et al, 1977). Nitrite oxidizes Mb to MetMb as it is reduced to nitric oxide (NO) through a series of reactions which initially results in the formation of nitrosylmyoglobin which provides the attractive pink to deep red color of cured meats (Fox, 1966), and with sufficient heating the protein (globin) is denaturized to form nitrosylhemochrome which is the final cooked cured meat pigment characterized with a pink-to-red color (Smith and Acton, 2001).

The meat industry has used carbon monoxide (CO) at levels of 0.3-0.5% in modified atmosphere, case-ready packages of beef, pork and lamb for over decade (Sorheim et al., 1999). Mb has a high affinity for CO forming carboxymyoglobin (CarboxyMb), a bright cherry red pigment on the meat surface. CarboxyMb is more stable than OxyMb; therefore, it is less likely to oxidize to MetMb during display (El-Badawi et al., 1964). However, due to safety issues only a few countries have approved the use of CO for meat packaging. In contrast to the Norwegian system, where the product remains in CO during display, the system currently approved for use in the United States requires the removal of packages from CO-containing atmosphere before display and sale (Hunt et al.; 2004). In 2001, the U.S Food and Drug Administration (FDA) affirmed CO as substance that is " Generally Recognized as Safe " (GRAS) for levels up to 0.4% in MAP systems (FDA, 2001).

During the formation of MetMb on the meat surface, and if the O2 concentration is not excessive, the meat will be reduced to OxyMb as a result of metmyoglobin reducing activity (MRA) within the muscle tissue (O'Keeffe et al., 1980-81 a, b, 1982). The MRA of muscle tissue is limited and once exhausted MetMb cannot be converted back to Mb (Gill 1991). Consequently, the meat becomes visually unacceptable when the level of MetMb reaches approximately 40% (Greene et al., 1971). Consumers may start discriminating purchase of fresh beef even at 10% MetMb levels (Hood et al., 1973).

Meat Discoloration

Fresh red meats cut and packaged for self-service retailing, undergo a discoloration, the rate of which is affected by: temperature, pH, lights, microbial activity, and modified atmosphere packaging. The surface of the meat turns brown as a result of oxidation of the bright-red OxyMb to MetMb (Snyder, 1964). Studies conducted by Brooks (1935) on hemoglobin showed that MetMb initially forms beneath the surface of meat. George and Stratmann (1952), showed that both heme pigments (Mb and OxyMb) autoxidize to MetMb most rapidly at partial pressures of O2 considerably lower than those present in air. As previously stated by Robach and Costilow (1961), deterioration of meat color is highly dependent upon the partial pressure of gases surrounding the meat.

Among the factors that can influence the partial pressure of oxygen of stored meat is bacterial growth that can reduce O2 in the environment. Psychrotrophilic, spoilage aerobic bacteria use O2 for respiration and thereby decrease the amount of O2 available for diffusion into muscle tissue. The penetration of O2 into the muscle is determined by the rate of oxygen diffusion and consumption (OCR) of oxygen (O'Keeffe et al., 1982). Feldhusen et al., (1995) demonstrated that the O2 partial pressure measured on a fresh cut surface was under 1kPa at 0.25mm below the surface, and the depth of O2 penetration was only 0.5mm. After five hours of exposure to O2, the measurement was only slightly higher: 2.7kPa at 0.25mm below the surface, and a 1.25mm total penetration depth. Bandall et al., (1972) explained that low O2 values were due to the relatively high activity of oxygen consuming enzymes present immediately post-mortem. However, after

3-13 days storage Feliburn et al., (1996) showed a clear increase in O2 partial pressure; 2.70 and 14.5 kPa for freshly cut surfaces, and 5 hours, respectively at the depth of 0.25mm.

Studies by O'Keeffe and Hood (1982) showed that the oxygen consumption rate of longissimus dorsi muscle falls to 30% of the initial rate by 2 days post-mortem, and penetration of O2 at the meat surface is related to both the degree of lightness and the saturation of meat color.

Beef has a relatively high Mb content compared to other meat products (Acton and Dick, 1986), and seems to be less color stable compared to other meat types exposed to similar conditions. In most beef color stability studies, objective color values decline significantly over time (Acton et al., 2004; Gatellier et al., 2001; O'grady et al., 1998; Formanek et al., 1998).

Other factors influencing the stability of Mb are temperature (Brown et al.; 1969; Lanier et al., 1978; Hood et al., 1980) and pH (Shikama et al., 1978; Chow et al., 1991). O'Keefe and Hood (1982) found OxyMb oxidation of beef increased with an increase in temperature and theorized that this oxidation of OxyMb could be caused an increased rate of various pro-oxidant reactions, decreased oxygen solubility, and/or increased oxygen consumption by tissue (Bendall and Taylor, 1972). Shikama and Matsuoka (1986) reported that the stability of OxyMb decreased with a decrease in pH in the acidic range (4-6.5). They suggested that the pH affects the configuration of the globin tertiary structure, which at low pH exposes the heme protein to more exposure to oxidation.

UV light is another factor influencing meat color stability, causing discoloration in many meat cuts. Kropf (1980) stated that display lightning could cause discoloration due to temperature increases on the meat surface. In general, light decreases color quality faster than products stored in the dark.

Bacterial growth will affect the color shelf life of meat and consumers acceptance for any packaged fresh meat. The spoilage flora of meat will usually be dominated by the bacteria which grow most rapidly under the storage conditions applied to meat (Gill, 1986), and may cause production of off odors and flavors and unacceptable appearance to the consumer. Under aerobic conditions, the dominant spoilage organisms are strict aerobic pseudomonads. When adequate glucose substrate is available, pseudomonads populations can reach about 100/cm2 before a total depletion of glucose occurs at which time pseudomonads will switch to amino-acid substrates, leading to metabolites that include amines characterized by putrid odors and flavors, (Gill 1982).

Modified atmosphere packaging (MAP) is one of the principal technologies used to inhibit rapidly growing pseudomonads. Slower growing organisms, notably lactic acid bacteria (LAB), psychrotrophic enterobacteria and Brochothrix thermosphacta, will then sometimes dominate the spoilage flora under MAP. (Gill, 1996). However, extension of color shelf life can be promoted by the use of MAP. Atmospheres combine oxygen (O2), carbon dioxide (CO2) and nitrogen (N2) to maintain the quality of fresh red meat, both from a microbiological and organoleptic standpoint (Luno et al., 2000). In high O2 + CO2 atmospheres, the growth of pseudomonads is inhibited by CO2. At 20% CO2 concentration, the growth rate of pseudomonads is approximately halved (Gill et al.,

1979). Under vacuum or oxygen-depleted atmospheres high in N2 or CO2, the anaerobic conditions slow growth of pseudomonads. On muscle and in exudates of pH< 5.8, anaerobic growth of facultative anaerobic enterobacteria and B.thermosphacta are totally inhibited. Grau, 1980). In an O2-depleted atmosphere with CO2, the growth of facultative anaerobes is further restricted. Growth of enterobacteria on high-pH tissue is prevented, as is the growth of B.thermosphacta at temperatures of 0˚C or below, (Gill and Harison, 1989) which substantially prolongs the storage life of high-pH products. Reveendran et al., (1993) compared aerobic and anaerobic methods for estimating bacterial numbers on fresh beef refrigerated under low O2/high CO2 MAP conditions or under vacuum. The results showed that at all temperatures tested (0, 2 and 4˚C), mean anaerobic plate counts were consistently greater than aerobic methods. The predominant bacterial groups found among 734 isolates stored at 0, 2 and 4˚C for up to 15 wk included lactic acid cocci (56%), staphylococci (41%), microccoci (2%) and Gram (-) fermentative rods (1%). These findings correspond with Nortje et al., (1990) where high numbers of staphylococci were found on fresh beef stored at 5˚C. According to many researchers, lipid oxidation has been implicated as playing a role in catalyzing color deterioration. Rancidity in meat involves the oxidation of unsaturated fatty acids, in particular polyunsaturated fatty acids (PUFA) (Kanner et al.; 1988). The content of PUFAs associated with membrane phospholipids (PLs) is believed to be the major factor for the development of off-flavor in meat (Moreck et al., 1989; Forgerty et al.; 1989) OxyMb and PL oxidation appears to be interrelated in meat (Green, 1969, Kanner et al., 1985). Dawson and Gartner (1983) reported that ferric heme pigments such as MetMb might

also act as catalysts, which can initiate proxidation. Yin and Faustman (1994) proposed that free radicals generated during lipid oxidation might initiate OxyMb oxidation. More recently, the products of lipid oxidation have been shown to contribute to OxyMb oxidation (Chan et al., 1997).

Modified Atmospheres Packages

Techniques used to alter the atmosphere surrounding food are known as MAP and include vacuum packaging. Modified atmosphere packaging is now used on a wide range of fresh or chilled foods, including raw and cooked meats and poultry. The key to success of these packaging methods for fresh meat is its ability to extend the keeping quality by reducing microbial growth while maintaining the attractive OxyMb bright-red color which consumer prefer (Taylor, 1985; Brody, 1989; Parry, 1993; Church et al., 1995). Modified atmosphere packaging of fresh meat normally uses mixtures of oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2); each gas plays a specific role in extending shelf life and maintaining a good appearance (Young et al., 1988). The main factors affecting the shelf life of MAP meat are summarized in table1.

Oxygen maintains the myoglobin in meat in the oxygenated form, OxyMb, thus giving the bright red color related to freshness by consumers. However, oxygen stimulates the growth of aerobic bacteria and inhibits the growth of strictly anaerobic bacteria. Low levels of oxygen <0.5% result in a color change in meat products (Church, 1993).

Carbon dioxide is the major antimicrobial component of MAP and is very effective for inhibiting Gram (-), aerobic spoilage bacteria such as *Pseudomonas* spp. CO_2 is also important in the MAP headspace for microbial control. In MAP systems for meat held at low temperatures, CO_2 dissolves in the aqueous phase of meat tissues and is also formed by tissue and bacterial respiration. Zhao et al (199) stated that absorption of CO_2 into fresh meat caused a decrease in headspace volume in MAP that can result in package collapse.

Nitrogen (N_2) is an inert gas, which has been used as a packaging filler to prevent pack collapse. As mentioned earlier, especially for fresh meat packed in high concentrations of CO_2, package collapse occurs because of the solubility of CO_2 in meat tissue. Nitrogen is used as filler gas because of its low solubility in water and lipid compared with CO_2. This prevents package collapse and allows the full effects of carbon dioxide addition to be achieved. (Gill et al., 1988).

Another potential modified atmosphere-packaging gas; carbon monoxide (CO) has been approved, and extends the red color in case-ready beef. CO binds strongly to myoglobin, maintaining bright red color and extending shelf life.

Table 1: The main factors influencing shelf life of MAP products.

Intrinsic factors	Extrinsic factors
Aw	Temperatures control at all stages
pH	Hygienic processing including implementation of HACCP procedures
Microbial flora:	
initial	Raw material quality
after processing	Finished product, i.e. combination of ingredients developing in package
Redox potential	Initial and final gas composition
Presence of naturally occurring anti-microbial compounds	Relative permeability of packaging film gases
	Gas to product ratio
Presence of spores	Gas purity, and pack design, e.g. circulation of gases

Another MAP option is vacuum packaging (VP) which presents some advantages related to the appearance of the package and color stability, such as; reduction in weight/volume loss due to dehydration, preservation of muscle color in its fresh state, and elimination of external contamination. However, VP still presents concerns about unattractive purge loss, which supports bacterial growth and can shorten shelf life. The most important criteria in VP systems are the type of film used and the degree of vacuum. Low rate oxygen transmission and low moisture transfer are recommended to inhibit the dehydration and prevent the re-entry of O_2 after evacuation of atmospheric air. As a result of an atmosphere depleted with oxygen, beef meat in VP has a purple-red color. Most consumers have become to prefer the bright red color which is an important quality factor to influence any purchase of meat. Vacuum skin packaging (VSP) is another method of vacuum packaging and results found a longer shelf life and better appearance for meat product compared with other types of packaging (Rice, 1994).

Another alternative for packaging for cut or ground meat is aerobic packaging, which allows O_2 to enter the package through the film to oxygenate the meat pigment.

Acton and Dick (1986) recommended low O_2 permeabilies for fresh meat packaging films. For instance, for shipping and storage of primal and subprimal, the recommended O_2 permeability of barrier type films was < 30-35cc/m^2/24hr(25°C, 0%RH), and for "hot processed" or post-rigor prepared ground beef for chub packs, the recommendation was a O_2 permeability < 50-60 cc/ml/m^2/24 hr (20ºC, 0%RH).

Extended storage life of retail-ready meat packs can be achieved by storage in a low oxygen master packaging system followed by aerobic storage (Isdell et al., 1999). In this system, packages are gas flushed in a nitrogen and carbon dioxide mixture then overwrapped in oxygen permeable film. These retail packages are further packaged in a master barrier pack flushed with CO_2 for storage up to 3-4 weeks. Finally, the packaged meat is removed from the outer master pack and aerobically displayed. Results showed that the success of this packaging method was dependent on the initial level of residual oxygen in the package. An O_2 concentration of 0.1% or less is recommended to prevent the formation of metmyoglobin on the steak surface (Jeremiah et al.1992, Ledward 1985). The use of an oxygen scavenger can help attain the needed oxygen-depleted atmosphere, however; consumers' acceptance of an oxygen sachet absorber is a limiting factor.

Moreover, it is known that both intrinsic (pH, water and fat content) and extrinsic (CO_2 partial pressure, headspace to meat volume ratio and storage temperature) factors affect the amount of CO_2 absorbed in meat. However, when applying high CO_2 partial pressures, the headspace volume will be reduced due to absorption of CO_2 into the meat. As stated earlier, this may result in package collapse. To ensure an attractive package

appearance, CO_2 must be used in an amount in excess of that needed to saturate the product. The optimum level of CO_2 has been reported to be 2 L/kg of meat (Jeremiah et al., 1996; Penney et al., 1993), 1–2 L/kg of meat (Gill et al., 1988) and 1–1.5L/kg of meat (Shay et al., 1987). The CO_2 solubility is also responsible for altering the foods-water holding capacity, which results in an increased drip loss (Davis, 1998).

Meat color measurement

Currently, two available methods for color evaluation are human sensory panels that are time consuming, and instrumental color analysis using colorimeters and spectrophotometers. The colorimeter method is based on numerical representations or quantifications in color systems such as Hunter and CIE tristimulus scales. In the popular L. a, b color space the lightness (L) axis in the center of the solid is calibrated 0-100, where 100 is absolute white. Positive a-values are red, and negative a-values (-a*) are green. Positive b-values (+b*) are yellow, and negative b (-b*) blue. The L-value is useful to determine the extent of product lightening (increase of L) or to darkening (decrease L), and the a-value is been useful to determine the change in pink-to-red Hue characteristic. Hue angle combines the a* and b* values to specify in terms of the angle between pure red (Hue angle= 0°) and pure yellow (hue angle= 90°) axes of color space. A higher hue angle corresponds to meat browning or " increase in yellowness over redness" within the meat color range due to the metmyoglobin formation.

A spectrophotometer method can be used to quantitate meat pigments and is based on reflectance to evaluate meat surface color without the need for pigment extraction and allows the pigment to be evaluated in its natural environment, the muscle.

Most myoglobin quantification methodologies are based on reflectance spectrophotometry and isobestic wavelengths. (Stewart et al., 1965). Although meat is considered opaque, light falling on the surface is partly absorbed and scattered due to tissue structure. Reflectance data usually are converted to K/S values by the Kubelka-Munk equation. Mancini and others (2003) suggested that K/S_{610} / K/S_{525} method was accurate and repeatable and could be used to quantify surface oxymyoglobin concentration and discoloration of ground beef.

Absorbance for myoglobin (Mb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb) is usually comparable at 525 nm and is referred to as the "isobestic" point on the spectrum and may be used to quantify the proportions of surface pigments (Bowen, 1949; Broumand et al., 1958). K/S ratios of 572/525 nm and 610/525 nm are commonly accepted as means for determining the percent of MetMb and OxyMb, respectively (Hunt et al., 1985; Domos et al., 1996). Results indicated that the K/S_{610}/ K/S_{525} method (direct method) was a good measure to predict ground beef surface OxyMb content (Mancini et al., 2002). OxyMb estimation calculated by direct method had a strong linear relationship with visual color indicating that direct OxyMb determination tracked red color deterioration (discoloration). Traditionally, discoloration during display has been estimated by an accumulation of MetMb rather than a loss of OxyMb. However, using the K/S_{610}/ K/S_{525} demonstrates decline of OxyMb content (discoloration) was equal to

or better than using $K/S_{572}/K/S_{525}$ to follow MetMb accumulation. This suggests that during display ground beef color stability may be tracked with $K/S_{610}/K/S_{525}$. As indicated by the low correlation between deoxymyoglobin (DeoxyMb) and visual color, discoloration of aerobically packed ground beef was not influenced by changes in DeoxyMb because of the low pigment content on the surface of fresh ground beef during display. (Mancini et al., 2003).

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EFFECT OF THE CO_2 GRINDING ON MODIFIED ATMOSPHERE AND COLOR SHELF LIFE OF GROUND BEEF

Introduction

Ground beef represents 44% of the total beef sold and its consumption in the United States accounts for about 3.2 billion kg/year (Anon, 1991). The increase in ground beef consumption led the meat industry to show more interest in methods to maintain color quality, which is an important factor associated with consumers' perception of meat freshness. By stabilizing the color in the final product, the meat industry could meet the customer's expectations.

Modified atmosphere (MAP) and vacuum packaging are used to extend product shelf life by inhibiting microbial growth. However, treating meat with 100% CO_2 during the grinding process may have a positive impact on extending the storage life. In addition, CO_2 is used in MAP of meat for its ability to inhibit the growth of microorganisms (Farber, 1991; Jerimiah, 2001; Zhao et al., 1994).

Processed meats such as ground beef are more susceptible to bacterial growth, drip loss and discoloration. The use of up to 100% CO_2 during the grinding processes may contribute to control the quality characteristics responsible for shortening meat shelf life. Knowing that CO_2 is highly soluble in muscle and fat tissue (Gill, 1988; Jakobsen et al., 2003), the delay of lipid and pigment oxidation is highly plausible under controlled storage conditions, especially CO_2 partial pressure, headspace to meat volume ratio and storage temperature (Jakobsen et al., 2002; Zhao, Well et al., 1995).

The objective of this study was to investigate the effect of injecting the CO_2 into meat during grinding using a modified grinder on the shelf life of ground beef under air-VAC (AV), Air- 100% CO_2 (AC) or 100% CO_2 –VAC (CV), 100% CO_2- 100% CO_2 (CC).

Material and Methods

Fresh Beef Meat

Fresh Sirloin beef was obtained 24 hrs before starting the experience from a regional supplier operating under USDA inspection. The meat was 96% lean ground beef, non-frozen and boneless, 4-5 days postmortem. The meat was transported from the supplier to Clemson University Laboratory within 10 minutes in insulated coolers with reusable ice blocks to provide temperature maintenance (approximately 7±1ºC). Meat was ground in air or CO_2 and was packaged in either vacuum or 100% CO_2. Meat shelf-life analyses began on different day for each of the 3 replications. A flow diagram of sample preparation and process is shown in Figure 1.

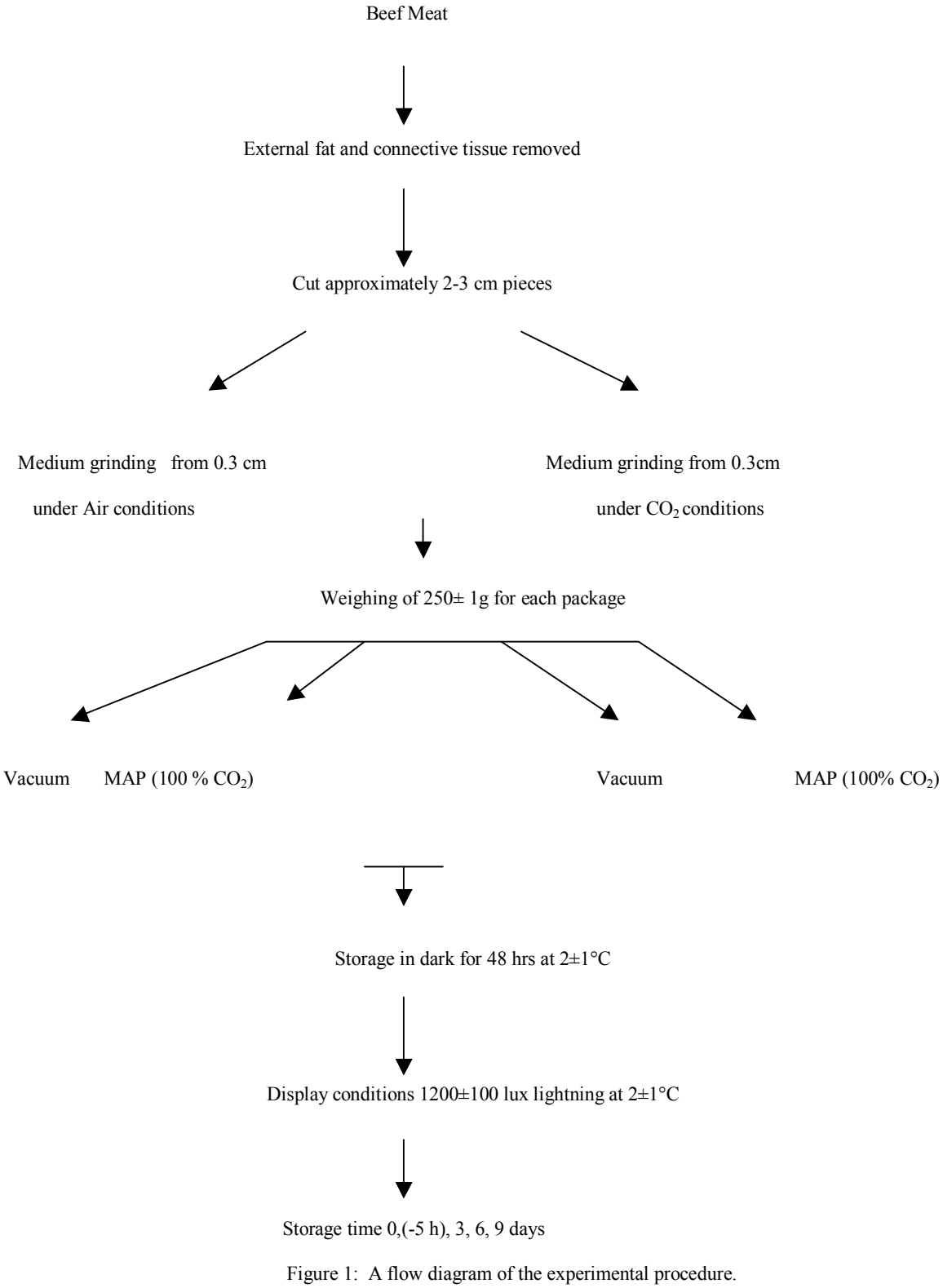

Figure 1: A flow diagram of the experimental procedure.

Sample Preparation

After removal from its package, external fat and remaining epimysial connective tissue (silver skin) were removed, meat was chopped into 2 to 3 cm pieces into a sterile container, then ground through a 0.3 cm diameter plate with a modified grinder (Toasters., High Torque Motor Model: TGR. 88, New Brunswick, Canada) with 4 injection holes drilled in grinding chamber in which a copper fitting was placed (Figure 2). The meat was divided in half, half of which was ground with CO_2 being injected during grinding and the other half being ground in air. For CO_2 meat, 3/16-inch diameter flexible manifold made from 4 flexible tubes were attached to a main CO_2 line. Pressure was maintained at 100 psi during grinding, and the meat was mixed with CO_2 gas by flushing the grinding chamber followed by holding ground meat in a barrier bag with a low OTR (20 cc/m^2/24h, 73˚F, 1atm, 0% RH) for 30 min. The CO_2 ground meat was flushed 3 times with CO_2. The bag was heat sealed (Midwest pacific impulse heat sealer, Model MP-12 Taiwan) and stored in the refrigerator for 30 min at 2±1°C to ensure that there would be no loss of gas during refrigeration. Air ground meat was treated the same as CO_2 - ground meat except no CO_2 was injected into the grinding chamber or into holding bags.

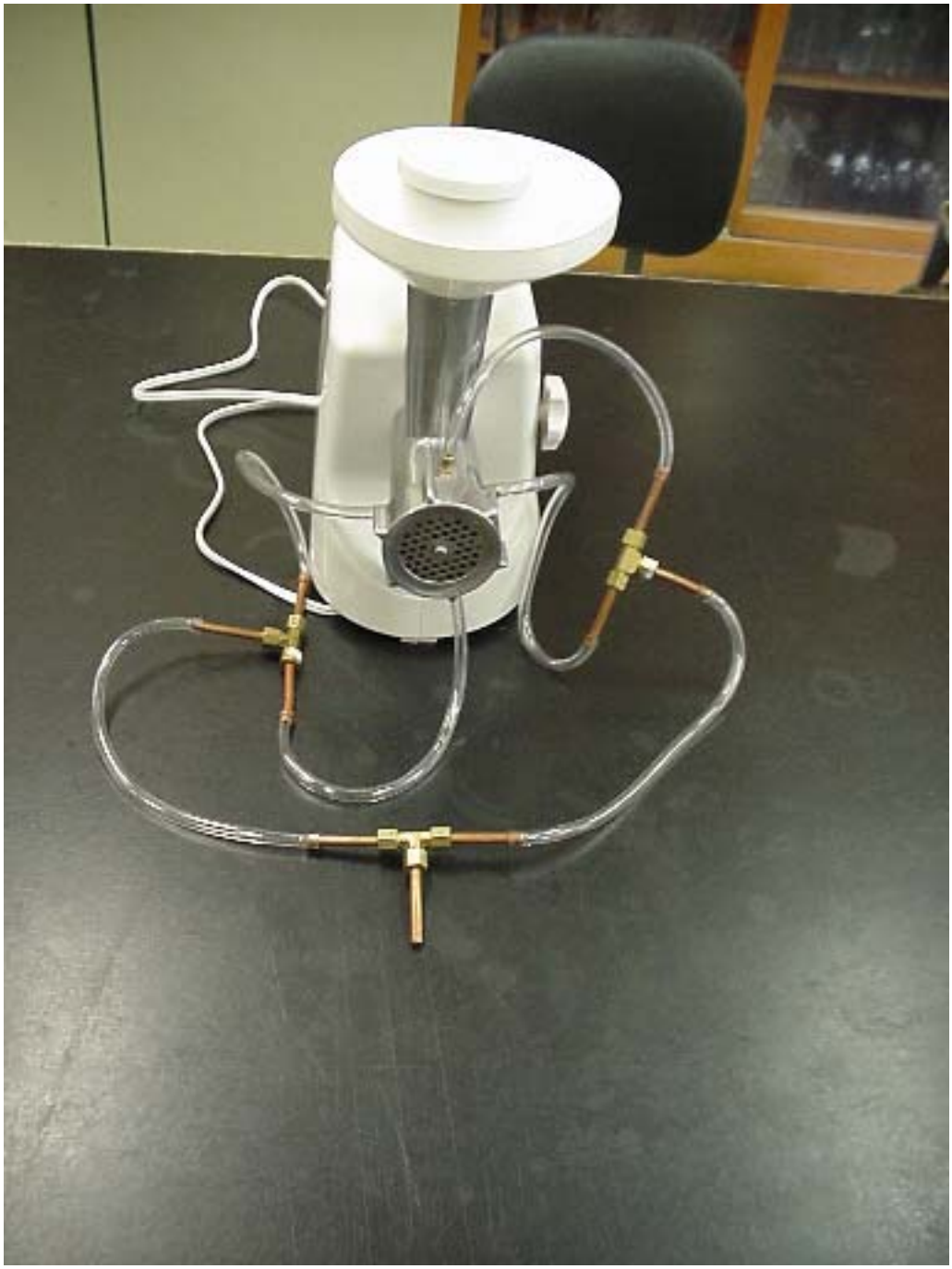

Figure 2: Picture of modified grinder machine

Packaging

Ground beef meat was packaged under the following conditions with the following package types (Table 2).

Table 2: Packaging conditions and package type used in the experiment.

Grinding conditions and packaging type		Package films
Air- vacuum	:[AV]	B- barrier film [1]
Air -CO_2(100%CO_2)	:[AC]	Lidded- Barrier foam tray [2]
CO_2-CO_2(100%CO_2)	:[CC]	Lidded-Barrier foam tray[2]
CO_2- Vacuum	:[CV]	B- barrier film[1]

[1]B-CNP-310 – Barrier cook-in film.

[2]B Multilayer barrier foam trays with Lid 1050-LLDPE

250 g of meat were weighed and placed in barrier foam trays (Cryovac, Sealed Air Corporation, Duncan, SC) prior to sealing.

Storage Packaging and Sampling

Packaging materials used in this study were provided by Cryovac., Sealed Air Corp., Duncan, SC. Trays used for packaging the ground beef were plain barrier polystyrene foam (C976 Sealed Air Cryovac, Duncan, SC) ($8^{3/4}$* $6^{3/4}$ * 15/8"). The trays were sealed using lid stock film (1050-LLDPE) (18.5" wide). Air-CO_2 and CO_2-CO_2 were packaged using a preformed tray modified atmosphere-packaging machine (Model Ross Jr No. S-3180, Robert Reiser & CO. Inc., Canton, MA 02021) after flushing with 100%CO_2 gas. Meat to gas headspace ratio in each package was3: 1 (i.e is 3 parts of gas and 1 part of ground beef). 250g of ground beef were packaged in barrier cook-in bag with low OTR equal at (20 cc/m^2/24h, 73˚F, 1atm, 0% RH) for 30 min, and were

performed by using Ultravac 2100 double chamber vacuum packaging machine (KOCH, Kansas City, MO). Vacuum 99%, vacplus 4 seconds and seal time 2 seconds.

Packaged ground beef meat was placed in boxes in the dark to simulate transportation for 48h at 2±1°C, and then refrigerated at 2±1°C under 1200±100 lux lighted display (Sylvania-Cool White Fluorescent lights) for up to 9 days. One package from each treatment was selected on sampling days of 0 (-5h), 3, 6, and 9, for headspace gas composition, color measurements, surface pigment discoloration and microbiological analysis.

Package Gas Headspace Analysis

A gas chromatograph (series 200, Gow-Mac Instrument Co., Bethlehem, PA) fitted with AllTech CTR-1 gas analysis column (catalog no. 8700, Alltech, Sanjose, CA) and thermal conductivity Detector (TCD) was used to determine the package headspace gases (CO_2, O_2, N_2). An integrator (Hewlett Packard, Willmington, DE) was used to plot chromatograms and calculate gas percentage from peaks areas. A 0.5ml package headspace gas sample was analyzed at each sampling interval. For vacuum packages, a sterile, high-density polyethylene ball (HB-02 practice golf ball, ZXS sports, Bentonville, AR) was placed in the bags to provide an air space for sampling. This was done by injecting a needle (syringe type) through a gas tight septum placed onto the package film surface.

Color Measurements

Four product packages from each treatment; [ground in air, packed in vacuum (AV); ground in air, packed in 100% CO_2 (AC); ground in CO_2, packed vacuum (CV); ground in CO_2, packed in 100% CO_2 (CC)] were selected and used for color determination at 3 day intervals during a total period of 2 days in dark and 7 days of lighted display. After opening packages, package film was allowed to contact the meat surface and press against the colorimeter head to carry out color measurements. Four readings per package were randomly recorded from the surface by a Minolta Model (DP-400 Chroma Meter, Japan). Also, care was taken to avoid any fat location during measurements. CIE L*(lightness), +a* (redness) and +b* (yellowness) were measured using an 8 mm aperture and illuminant C after calibration with a standard white plate (CIE L*= 97.18, a*= -1.10, b*= 1.89).

Spectral Analysis

To measure surface reflectance for pigment concentration calculations of the MAP ground beef, one package was removed from the display refrigerator, inverted, and placed on clean lab table. The bottom portion of the barrier foam tray was cut with a knife and removed. A rigid rectangular plastic plate was placed against the bottom of the ground beef in the package and used to press the top surface of the meat against the film lid. Sufficient pressure was applied to ensure full contact between meat surface and lid film and care was taken to avoid expressing fluid from the meat. Care was also taken to measure lean meat surface color, while large fat particles, if present, were avoided. The package was then held under slight pressure and placed at the 2.54 cm diameter aperture

on a HunterLab UltraScan XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, Virginia). Reflectance scan (360-750nm) was measured at 4 different loci on the meat surface. Each day (0, 3, 6, 9), surface color characteristics of one package from each treatment was measured and recorded. For vacuum packages, the % reflectance was read directly through the bag.

The spectrophotometer was calibrated with a standard white ceramic tile each analysis day. In all pigment conversion methods, reflectance (R) at wavelengths 610, 572, 525 and 474 nm were determined (wavelengths 572,525 and 474 nm were determined by linear interpolation) and converted to K/S ratios (Stewart and others, 1965) using the Kulbelka-Munk equation [$K/S = (1-R)^2 /2R$] where K=absorption coefficient and S=scattering coefficient.

Surface Pigment Determinations

Proportions of myoglobin pigment forms were determined using a slightly modified method of AMSA (1991). A reference sample with 100% MetMb at the meat surface was obtained by keeping the meat at room temperature to fully oxidize (100% surface discolored) the ground beef and spectral data were made from 4 different meat surface locations. Reference values for 100% OxyMb were obtained by placing ground beef in a bag flushed 3 times with O2, after which the bag was sealed and placed under refrigeration for 30 min at 2±1°C. Another flushing with O2 was made just before the reflectance values were read. Meat surfaces were scanned to obtain reflectance spectra for each pigment at its highest achievable levels of OxyMb, Mb and MetMb.

Reflectance and K/S Spectral Analysis

Percent reflectance between 360-750 nm was recorded in 10 nm intervals simultaneously with each color measurement. Reflectance values were converted to K/S_{474}/ K/S_{525}, K/S_{610} / K/S_{525} and K/S_{572}/ K/S_{525} to indicate the increase or decrease of %Mb, OxyMb and MetMb for the MAP ground beef. (Stewart et al., 1965; Hunt et al., 1985; Gill et al., 1995). The surface reflectance scans for each packaged sample were collected at 3-day intervals throughout the 9 days of storage. Individual sample results were averaged and converted into reflectance attenuance (A= Log10 1/R), analogous to calculation of absorbance from transmittance, as described by Shibata (1962) and Swatland (1983). Mb, OxyMb and MetMb percentages were calculated using the following Kryzywicki (1979) equations.

$$\% \text{ MetMb} = (1.395 - [(A_{572} - A_{720})/(A_{525} - A_{720})]) * 100 \quad (1)$$

$$\% \text{ Mb} = 2.375 * [1 - (A_{473} - A_{720})/(A_{525} - A_{720})] * 100 \quad (2)$$

$$\% \text{ OxyMb} = 100\% - \% \text{ MetMb} - \% \text{ Mb} \quad (3)$$

Microbial Analysis

Eleven grams of ground beef were taken from the meat surface and placed in a sterile stomacher filter bag (Seward, Model 400 Bags 6141), with 99 ml of 0.1% peptone solution (Difco^{TM} , Bactopeptone, Bacton, Dickison & Company, MD, USA 21152) and were blended in a stomacher blender (model 400, 6041/STR, Seward Limited, London, UK) for 1 min at 230 rpm. Appropriate serial dilutions for total plate count (TPC) were prepared and plated (Petri Dish, Polystyrene sterile, 100x15mm, VWR International, Suwanee, GA 30024) using agar media (Difco, Plate Count Agar, Becton, Dickison & Company, MD, USA 21152) and incubated for 48 hours at 37°C. Dilutions with 30-300 colonies were counted and converted to log numbers of colony forming units (CFU)/g of ground meat. Each treatment was analyzed in duplicate at 3 day intervals up to 9 days of storage.

Statistical Analysis

A randomized complete block (RCB) with replication as the blocking factor was used as the experimental design. The treatment structure was factorial with three treatment factors: grinding condition, package type, and day of storage. The experiment was replicated on three different time periods using three different lots of fresh beef. Analysis of variance was used to analyze the data and the LSD multiple comparison procedure was used for mean comparison. The p-values for the models and the predictions lines were obtained by Proc Mixed using SAS 9.1.

Results and Discussions

CIE L* color values (Lightness)

Overall, the L* values did not differ (P>0.05) between treatments during storage, whereas L* values for grinding conditions under CO_2 were affected from 0 to 3 days (P<0.05) (Figure 3). Pooled over time, L* values for AC and AV packages did not differ (P>0.05). However, for AV treatment there was evidence of linear trend over time (P<0.05). At day 3, meat from CC treatment had higher L* values (44.19) than AC, AV and CV (43.19, 42.89, 41.93, respectively).

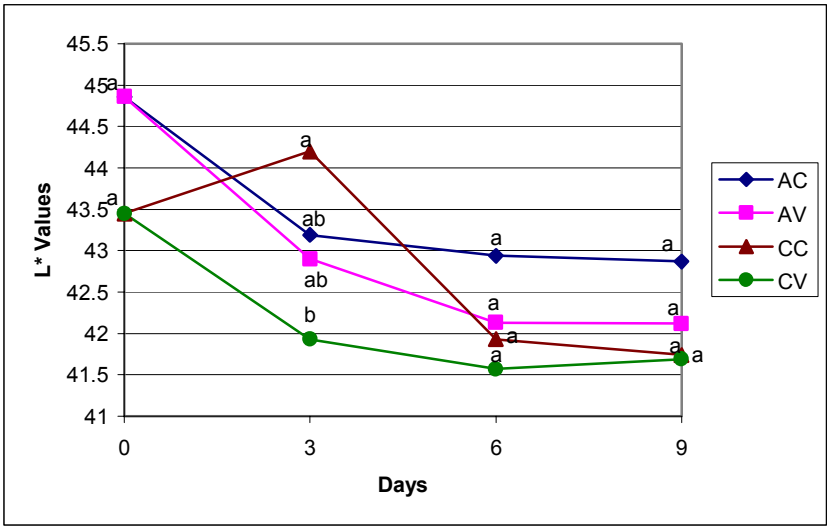

Figure 3: Least Square Means for L* values for grinding conditions x type of package x day interaction (P<0.0001)

AC = Air/CO_2; AV = Air/Vacuum, CC = CO_2/CO_2; CV = CO_2/Vacuum.
*SEM is the Standard Error of the Least Squares Means (SEM = 0.94, n=12)
[a-c] means within days with different letters are significantly different (P<0.05).

CIE a* values (Redness)

a* values ("redness") at 2±1ºC remained constant under vacuum packaging, but decreased in air-ground, and CO_2-ground MAP beef from day 0 to day 3. (Figure 4). At day 3, mean a* values for ground beef under air and CO_2 grinding conditions were 16.94 for AC, and did not differ from AV, CC, and CV with 17.02, 16.97 and 17.18, respectively. The lack of oxygen in vacuum package generally maintained the ground beef with lower a* values compared to MAP packages.

AC and CC meat did not differ in a*values from CV and AV treatments by day 3, but a* values for MAP packaged (AC and CC) increased significantly during display from day 3 to day 6 with higher a* values due to the change of Mb (dull color) to OxyMb (bright red color) in the beginning of display period. This change continued for the AC treatment until day 9, but not for CC- meat from day 6 to 9. As Jeremiah and Gibson (2001) reported, in general, a* values decrease over storage display as was observed only from day 0 to day 3 for all treatments. In addition, under air grinding conditions a* values for meat in MAP packages (the barrier foam tray) was higher than a*values for meat in vacuum packages (barrier bag), (p-value = 0.0434 and p-value= 0.0237, respectively). Steady decreases in L* values after 3 days coupled with simultaneous decreases in a* values (redness) reflects a visual surface color shift from redness to brown, which was observed in our study between day 0 and day 3. However, lower L* values and higher a* values indicate a better meat color (Manu-Tawiah et al., 1991), which is the case for CC at day 6. In addition, a* values increased for AC from day 3 to 9 and CC from day 3 to 6 demonstrating that CO_2 did not allow the ground beef to fully

oxygenate. There was an initial decrease in a* values of 3 units for AC and 0.5 units for CC from day 0 to day 3, before a substantial increase of approximately 2 units for AC and CC from day 3 to 6. The general decrease in meat a* values during display (Jerimiah et al., 2001) was not found in the present study for CC treated meat between day 3 and 6, and for AC- meat between day 3 to 9.

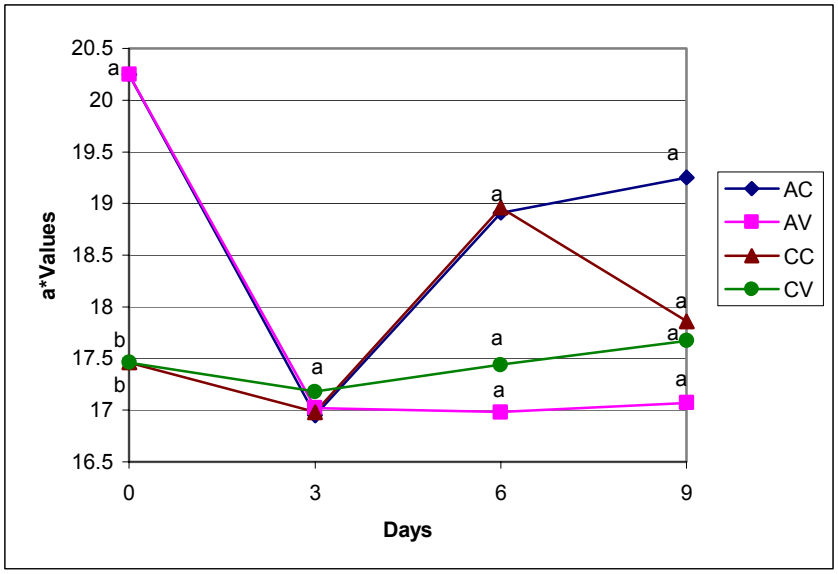

Figure 4: Least Square Means for a* values for grinding conditions x type of package x day interaction (P<0.0001).
AC = Air/CO_2; AV = Air/Vacuum, CC = CO_2/CO_2; CV = CO_2/Vacuum.
*SEM is the Standard Error of the Least Squares Means (SEM = 0.81, n=12)
[a-c] means within days with different letters are significantly different (P<0.05).

CIE b* values (Yellowness)

During storage, b* values decreased (P<0.05) for vacuum packaged meat. Pooled over 6 days, b* values differed between all package types. However, for AV and CV treatments b* values did not change (P>0.05) during the 3 to 9 day storage period. For AC treated meat, a subsequent increase in b* values occurred from day 6 to 9. Moreover,

CV and AV had a noticeable decrease in b* value from day 0 to 3 of about 7 units and 6.5 units, respectively before they stabilized over the remaining display period. From day 0 to day 3, the b* values decreased for all four treatments. (Figure 5)

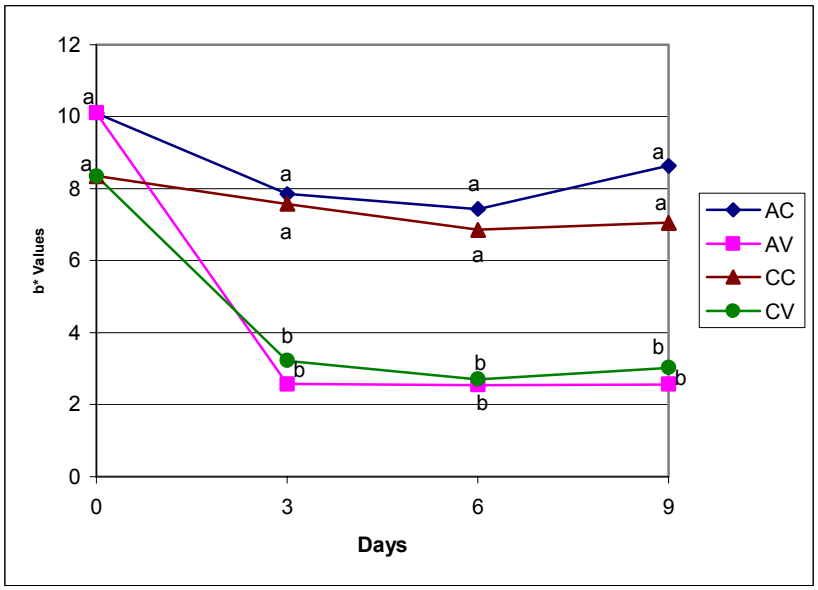

Figure 5: Least Square Means for b* values for grinding conditions x type of package x day interaction (P<0.0001).
AC = Air/CO_2; AV = Air/Vacuum, CC = CO_2/CO_2; CV = CO_2/Vacuum.
*SEM is the Standard Error of the Least Squares Means (SEM = 0.66, n=12)
[a-c] means within days with different letters are significantly different (P<0.05).

Hue* values

An increase in hue angle between 0˚ and 90˚, corresponds to a "blending of yellowness" or "loss of redness" likely due to MetMb formation in fresh meat. It was noticed that grinding conditions and package types affected hue (P<0.05), whereas hue angle did not change during storage for vacuum packages meat (P>0.05). Both AV and CV maintained the best color (lower hue angle) over the display period. (Figure 6). In contrast, CC and AC had higher hue angles reflecting an increase in the percentage of

MetMb. The hue angle for day 0 was the highest for any day and neither grinding treatment (Air/CO_2) gave an advantage in stabilizing hue between days 3 through day 9. The only differences in b* values seemed to be due to package atmosphere (P<0.001).

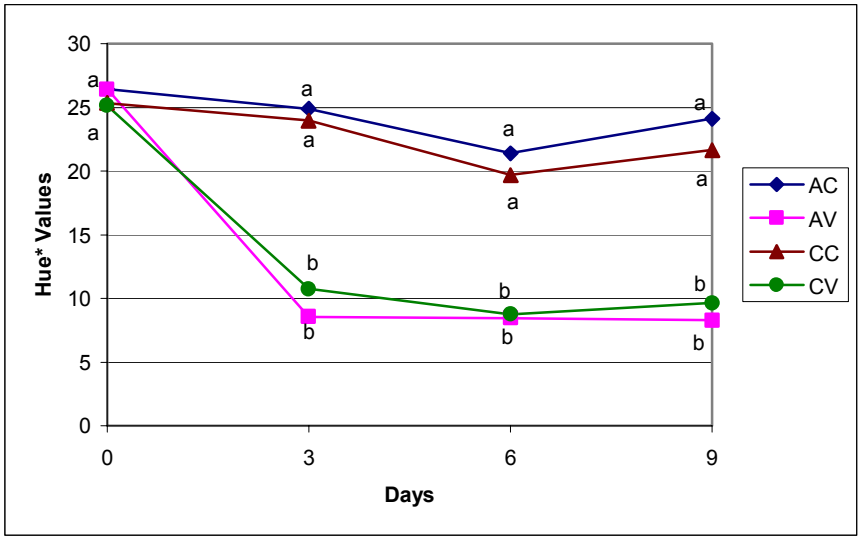

Figure 6: Least Square Means for Hue* values for grinding conditions x type of package x day interaction (P<0.0001).
AC = Air/CO_2; AV = Air/Vacuum, CC = CO_2/CO_2; CV = CO_2/Vacuum.
*SEM is the Standard Error of the Least Squares Means (SEM = 1.63, n=12)
[a-c] means within days with different letters are significantly different (P<0.05).

Chroma* values

Chroma (saturation) (C*) values is calculated from a* and b* values, and is a measure of color intensity where the higher values represents more intense color. From day 3 through day 6, AC and CC had the higher C* values than AV and CV. (Figure7). Taylor et al. (1980) found that meat samples with C* values above 20 were regarded as acceptably bright red by consumer panels. AC maintained C* above 20 after 6 and 9 days, and for CC at day 6, indicating that these 2 treatments had good impact on the meat

color stability. For CO_2- injected meats, there was a difference in C* due to package types on day 6 ($P<0.05$). In contrast, C* values for AV and CV at day 0 were high, but dropped thereafter, indicating poor color stability. According to Taylor et al., (1990) an average of 16.81 C* values, signifying the presence of a level equivalent to between 20 and 40% MetMb formation on the surface of the ground beef. The low C* values (saturation) for beef samples under vacuum at day 9 have a typical purple-red color of vacuum packaged beef, and thus remained almost constant during storage.

Hunt et al (1991), reported that brown colors are difficult to measure instrumentally, and for meat, it is highly recommended to measure the lack of redness. Therefore L*, hue and C* values have been used to measure loss of redness and beef under MAP which had higher a* and C* values and this effect was more evident with increasing storage time. In the current research color parameters were more constant over time for CC and AC treatments while CV and AV showed a decrease in CIE L*, a*, b*, hue* and C* values for the first 3 days followed by stable values over days 3 to 9. Other studies have demonstrated that muscle with high color stability have increasing in L, a, b values during storage (Moore et al., 1991, and Hernandez et al., 1999) which agreed with our findings. Insausti et al., (1999) stated that L*, a*, b*, h* and C* physical variables increased during the first 5 days of storage and in both vacuum packaged and MAP packaged meat. Higher chroma values for AC and CC meat packages represent maintenance of color intensity (Dawson and Acton, 1995), due to the stabilizing of product redness (+a*) by OxyMb (Pierson et al., 1970).

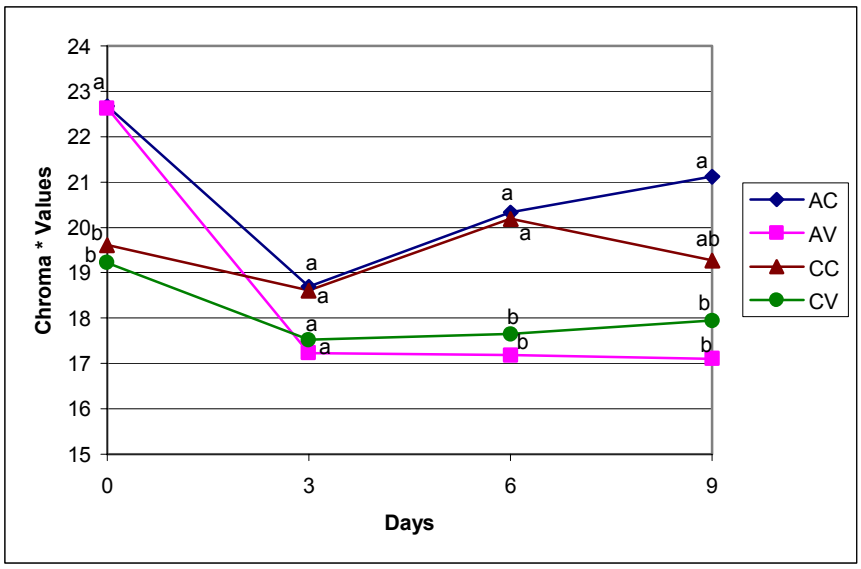

Figure 7: Least Square Means for Chroma* values for grinding conditions x type of package x day interaction (P<0.0001).

AC = Air/CO_2; AV = Air/Vacuum, CC = CO_2/CO_2; CV = CO_2/Vacuum.
*SEM is the Standard Error of the Least Squares Means (SEM = 0.91, n=12)
[a-c] means within days with different letters are significantly different (P<0.05).

Microbial Analysis

Initial microbial count (total aerobic count) of ground beef was 10^4 CFU/g. As expected, during storage, bacterial populations increased (P<0.05) for all types of packages at each sampling day. There were differences in total counts between days 3 through 9 (P<0.05). CC treated meat had lower microbial populations for day 3, 6 and 9 compared to other treatments, supporting the effect that the inhibition of many spoilage bacteria is proportional to the amount of dissolved CO_2 in the product (Biorn et al., 2006). At day 9 total viable counts of ground beef under CC was less than 10^6 CFU/g, resulting from the bacteriostatic effect of CO_2. The inhibitory effect of CO_2 on aerobic spoilage bacteria is well documented (Boran et al., 1970; Huffman et al., 1975; Ingram,

1962; Kraft and Ayres, 1952; Ordal, 1962). Meat packaged in 100% CO_2 had lower total aerobic populations on day 9 compared to vacuum packaged meat, regardless of grinding treatment.

CO_2 inhibited the bacterial growth and throughout the storage period (9 days), ending at 6.25 log CFU/g, 6.35 log CFU/g, 7.38log CFU/g and 7.44 CFU/g for CC, AC, AV, and CV, respectively after 9 days storage. (Figure 8) In summary, fresh ground beef under CC and AC stored for 9 days of retail display had lower microbial counts compared to CV and AV.

Bacterial activity is another factor in pigment changes in fresh meat. Meat spoilage was also related to discoloration of meat (Robach and Costelow, 1961). Maintaining the low microbial populations will slow meat discoloration since one effect of bacteria is reducing oxygen tension at the meat surface tissue. Butler et al., (1953) stated that the rate of MetMb was greatest during the bacterial logarithmic growth phase. In contrast, Faustman et al., (1990) concluded that color change was not necessarily related to the level of microorganisms in meat.

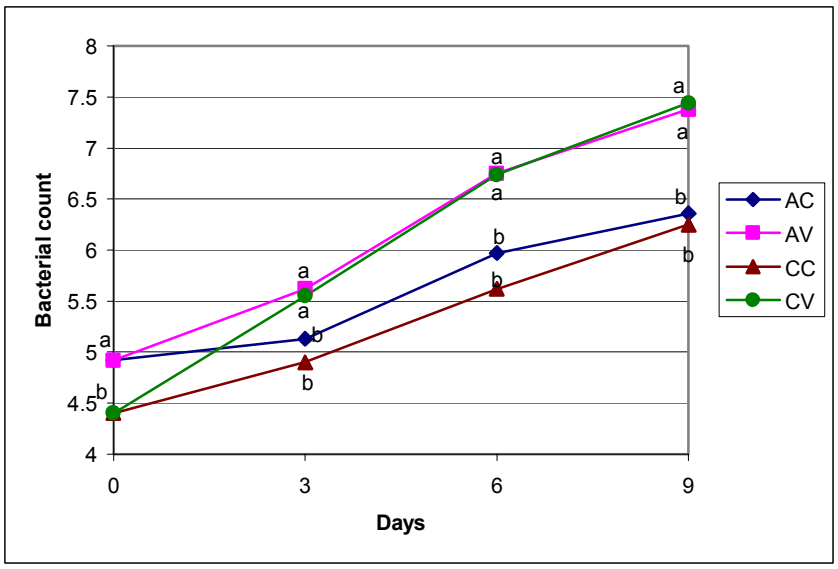

Figure 8: Least Square Means for Total Bacterial Count for grinding conditions x type of package x day interaction ($P<0.0001$).

AC = Air/CO_2; AV = Air/Vacuum, CC = CO_2/CO_2; CV = CO_2/Vacuum.
*SEM is the Standard Error of the Least Squares Means (SEM = 0.18, n= 6)
[a-d] means within days with different letters are significantly different ($P<0.05$).

Head Space analysis

Carbon Dioxide (CO2)

CO_2 package headspace concentration decreased for AC between day 0 and day 6, then increased from day 6 to 9. (Table 3). The reduction of CO_2 is mainly caused by the high solubility of CO_2 in both muscle and fat (Gill, 1988; Jackobson & Bertelsen, 2003). The absorption capacity is related to biological factors, i.e, water and fat content (Gill, 1988), but is also impacted by packaging and storage conditions, specifically CO_2 partial pressure, headspace to meat volume ratio and storage temperature (Jackobson & Bertelsen, 2002; Zhao, Wells & MC Millin, 1995). In addition, high CO_2 concentration in the headspace of packaged meat would be absorbed by the muscle and fat tissue until

saturation or apparent equilibrium (Bush, 1991). Gill and Penney (1980) reported that absorption of CO_2 also causes a decrease in headspace volume in MAP resulting in shrunken or small deflation of the package which was observed in our study more in the AC treatment.

The package headspace CO_2 increase for AC treated meat from day 6 to 9 corresponded to a decrease in O_2 percentage. This was explained by Daun et al (1971) that part of the increase in CO_2 in MAP results from the interactions of solubility, temperature and headspace volume. Since mitonchondria respire for no longer than 144 hrs post-mortem (Cheah and Cheah, 1971), bacterial respiration may be responsible for the conversion of O_2 to CO_2 in packages initially ground under air conditions and packed in 100%CO_2. Johnson (1974) and Gardner et al (1966) have previously suggested that bacteria convert O_2 to CO_2 in MAP. The CC treatment did not present any changes in CO_2 % ($P>0.05$) through storage since this meat/headspace was likely to be nearly CO_2 saturated. The CO_2 mean values for CC package headspace at day 9 were 94.14%. Although, Pohja (1967) and Ledward (1970) determined that high concentrations of CO_2 could discolored meat, which was observed during the 2 days storage in the dark in our study. However, the maroon-brown color that was observed during lighted display supporting the results of Taylor (1972) who reported that 50-80% CO_2 is often found in the residual air spaces of vacuum packaged meat with no detrimental effect on color.

Headspace analysis for empty container flushed with 100% CO_2 for each sampling day was conducted to determine package integrity and that headspace gas concentration changes were due solely to meat/gas interactions. The results (table B2)

showed no significant changes (P>0.05) during storage period for CO_2, O_2 and N_2. The CO_2 permeation of the empty tray (lid film) at 2 ± 1º C showed no major diffusion out of the trays (P>0.05) throughout 9 days storage. According to Henry's law, the decrease of CO_2 in headspace would lead to a decrease of dissolved CO_2 in the products.

Table 3: Change in mean CO_2 concentration for AC and CC treatments throughout the storage period.

Time (Days)	AC (%)	CC (%)
0	96.41[a]	96.41[a]
3	92.01[a]	93.69[a]
6	78.03[b]	93.83[a]
9	88.97[a]	94.14[a]

AC: Air grinding/ 100 % CO_2 MAP
CC: CO_2 grinding/ 100% CO_2 MAP
Standard error of mean (SEM = 2.785, n=3)
[a-b] means with different letters for each column are significantly different (P<0.05).

Oxygen (O2)

There was an increase in O_2 concentration for AC from day 0 to day 6 after which O_2 levels decreased from day 6 to 9. The relative decrease in O_2 % headspace could be due to muscle tissue respiration at high pH and the consumption of O_2 during bacterial metabolism. The low level of O_2 for AC treatment during storage day 3 and 6, respectively, about 0.56% and 1.14%, may have contributed to discoloration and increases in MetMb. There were no significant changes in CC treatments for O_2 % (P>0.05) from day 0 through end of storage period, (Table4), which may be due to saturation of the meat tissue with CO_2 during grinding. No difference in the amount of O_2 in headspace of empty tray (P>0.05) was observed, indicating that the film

permeability did not play a role in any package gas headspace concentration during 9 days of storage.

Table 4: Change in mean O_2 concentration for AC and CC treatments throughout the storage period.

Time (Days)	AC (%)	CC (%)
0	0.28^{b}	0.28^{a}
3	0.56^{b}	0.00^{a}
6	1.14^{a}	0.00^{a}
9	0.00^{b}	0.06^{a}

AC: Air grinding/ 100 % CO_2 MAP
CC: CO_2 grinding/ 100% CO_2 MAP
Standard error of mean (SEM = 0.372, n=3)
$^{a-b}$ means with different letters are significantly different ($P<0.05$).

The remaining gas was composed of nitrogen which is inert and does not alter meat color.

The amount of CO_2 absorbed by beef under CO_2 and air grinding was estimated by putting 12g of CO_2 ground beef and 12g of air ground beef in different vial and maintained at room temperature for 2 hrs. The solubility of CO_2 in beef and the predicted values of CO_2 dissolved in meat were measured with a gas analyzer. These results showed no changes between the CO_2 and the air in the headspace vials ($P>0.05$). This seems that the use of the CO_2 grinding had no significant effect on the increased CO_2 absorption compared to the air grinding even though there were already plenty of CO_2 to saturate the fresh ground beef under the CO_2 grinding process.

Surface Reflectance and K/S Spectra

Oxymyoglobin (OxyMb)

Surface reflectance spectra for ground beef of the four packaging treatments during 9 days storage are shown in Figure A1 to A4. Oxymyoglobin content was measured using the ratio of surface reflectance at 610 nm to surface reflectance at 525 nm, and lower ratios indicate higher levels of OxyMb. Two peaks between 545 and 575 nm where noticed for all treatments at day 0. However, CC and AC presented a typical spectral curve by 9 days display, but peaks at 545 and 575 nm started to flatten between day 6 and 9. (FigureA7- A8)

Van den Oord and Wesdrop (1974) reported that the threshold for acceptable color on beef was approximately 50% of OxyMb. During display only AV and CV had high $(K/S)_{610}/(K/S)_{525}$ values from day 3 to 9 compared with CC and AC (Fig A9), which may be related to incomplete oxygenation. Day 6 for CC treatment represents the lowest $(K/S)_{610}/(K/S)_{525}$ values (0.32units) with a slight increase (0.38units) on day 9 indicating a moderate loss of OxyMb over the course of retail display.

The visual appearance of meat changed from an initial bright red to dull red color by day 9 for AV and CV treatments. A day 6, peaks at 540 and 575 nm indicated mainly OxyMb at the meat surface. The peaks flattened by day 9 and a small band at 630 started to appear. (Figure A8).

For OxyMb content, the interaction of grinding conditions x package type x day treatment (p-value = 0.043) were significant. As $(K/S)_{610}/(K/S)_{525}$ increased (Figure A9), surface OxyMb concentration decreased, which supported the findings of Van den

Oord et al., (1971). Values for MAP meat appeared to be in acceptable range between 0.31 units and 0.38 units $(K/S)_{610}/(K/S)_{525}$, which corresponds to 45% and 50% OxyMb from day 3 to 9. In contrast, meat in vacuum packages (barrier bag) reached an average of 0.41 $(K/S)_{610}/(K/S)_{525}$ which corresponds to 0% OxyMb at day 9. This can be explained by the low OTR of the package material. However, MAP type package had the highest OxyMb % due to the possible presence of residual O_2 entrapped during packaging process allowing an oxygenation of surface pigment producing uniform cherry red color until oxidation begins to change the surface color to a brown tan color.

Metmyoglobin (MetMb)

Metmyoglobin content of meat under CO_2 grinding did not differ from meat ground in air at any sampling time ($P>0.05$). However, there was difference between the 2 types of packages CC and CV (p-value = 0.041). The ratio of surface reflectance at 572 nm to reflectance at 525 nm is an important measure to evaluate the total amount of MetMb formed on the surface of ground beef package. $(K/S)_{572}/(K/S)_{525}$ values at day 0 for CC, AC, CV and AV (1.12, 1.20, 1.12 and 1.20), respectively. As MetMb accumulates, through storage, the ratio between reflectance values decrease, indicating the presence of higher level of MetMb on the surface of ground beef. In our study, CC treatment decreased in $(K/S)_{572}/(K/S)_{525}$ ratios between days 6 to 9. (Figure A10).

For all four treatments, there were no substantial losses or decreases in MetMb except for CC treatment with 0.09 units between days 6 to 9. MetMb levels of AV and CV treatments increased over the retail display period, especially from day 0 to 3 before

they stabilized until the end of storage. In summary, $(K/S)_{572}/(K/S)_{525}$ for ground beef at day 9 was between 1.20 and 1.27 which were observed on day 9 in our study under display conditions indicating that an acceptable percentage (24 to 26%) of metmyoglobin is present on day 9 and that little discoloration has occurred.

Stewart, Zisper, and Watts (1964b) reported a linear relationship between $(K/S)_{575}/(K/S)_{525}$ and MetMb %. They published that $(K/S)_{575}/(K/S)_{525}$ values equal to 1.40 and 1.30 equaled to approximately 0% and 13% MetMb, respectively. Additionally, they reported that $(K/S)_{575}/(K/S)_{525}$ values equal to 1.20, 1.10, 1.0 and .90 correspond to approximately 21, 33, 46 and 59% MetMb. Our results $(K/S)_{572}/(K/S)_{525}$ values for the present study were between 1.12 and 1.27 which correspond between 20 and 30% MetMb supporting the conclusion that meat was still in a consumer acceptable range for appearance and attractiveness.

Myoglobin (Mb)

There was no interaction between package conditions x package type x days for $(K/S)_{474}/(K/S)_{525}$ ratios ($P>0.05$). There was a difference in Mb content over time for AC and AV (p-value < 0.001). Decrease in $(K/S)_{474}/(K/S)_{525}$ corresponds to a higher Mb %. The $(K/S)_{474}(K/S)_{525}$ for ground beef shifted for AV and CV recording the lowest K/S ratios (0.54) at day 9 (Figure A11), corresponding to 78 % Mb. Obviously, the type of package played an important role in Mb content for MAP type package (Barrier foam tray) where a slight amount of oxygen might have permeated the packaging film oxygenating Mb. In contrast, the vacuum package (barrier bag), created an anoxic

environment maintaining high levels of Mb in the meats not permitting oxygenation of the pigment. According to Mckenna (2003), Mb content decreased overtime, which support our findings since Mb content (after an increase in from day 0 to day 6) decreased throughout the lighted display period from day 6 to day 9, for AV, CV, AC and CC.

Surface pigments and total heme percentage

Relative surface pigment concentration of the ground meat under CO_2 / Air conditions packed with 100% CO_2 MAP or vacuum packaged are based on reflectance were determined by the equation of Krzywicki (1979) (Table 5). The grinding conditions x package type x day interactions were not significant ($P>0.05$) for OxyMb, Mb and MetMb. However, they were changes between type of packages MAP or vacuum (p-value = 0.0070, p-value = 0.0002) during 9 days for Mb and OxyMb, respectively. For fresh ground beef in AC and CC treatments OxyMb content initially decreased probably due the simultaneous increase in Metmyoglobin Reducing Activity (MRA) (Echevarne et al., 1990) between day 0 to 3. Normally an increase of OxyMb concentration is predictable due to exposure to O_2 during storage periods of 3-4 days post-mortem. Later, OxyMb content increased between 6 to 9 days, while OxyMb % remained in an acceptable level (45- 48%) at day 9. (Figure A12) According to Ordonez and Ledward (1977), low relative amounts of Mb and MetMb, and high relative amounts of OxyMb, are to be expected in meat stored after processing in conditions rich in O_2 which support our findings for all treatments at day 0.

Surface MetMb contents revealed no marked difference (P>0.05) from day 0 to 9 with the exception of AV and CV treatments which differed at day 0 (p-value = 0.0194). In the present study, MetMb % decreased from day 0 to 9, 3% to 7 % and remained in the range of 24% to 31% during display. (Figure A13)

Greene et al. (1971) reported that 40% MetMb resulted in meat rejection by consumers. Solberg (1970) stated 50-75% MetMb gave meat an undesirable brown color. In this experiment, all treatments would be accepted if we considered > 40% MetMb as the rejection point (Stewart et al., 1965). Nevertheless, vacuum packaged maintained a more stable level of MetMb over time due to the oxygen-depleted atmosphere and a low OTR bag. The meat was effectively removed from exposure to oxygen at the time of packaging. The slight decrease in MetMb of about 1% and 7 % for AV and CV, respectively, may be reconverted to Mb depending on the capacity of metmyoglobin reduction activity (Hood, 1980).

Myoglobin content increased over time in the present study for all four treatments from day 3 to 6 and decreased later from day 6 to 9, which does not agree with Mckenna (2003) who stated there is Mb degradation during storage. The barrier film does not permit O_2 across to transform Mb to OxyMb and maintains a high level of Mb % in vacuum packages. This explains the decrease of OxyMb from 55% to 0% and 35% to 0.5% for AV and CV, respectively. (Figure A12)

Table 5: Surface pigment concentrations of different treatments of ground beef during lighted display. Pigment concentrations were calculated using Krzywieki (1979).

	Time (days)	AC	CC	AV	CV
% Mb	0	16.80	17.63	16.80	17.63
	3	19.13[a]	19.26[a]	71.10[a]	74.93
	6	29.46[b]	33.10[d]	78.06[c]	76.90[b]
	9	27.13[e]	28.96[h]	63.83[g]	55.40[f]
	SEM	7.46	7.46	7.46	7.46
% OxyMb	0	55.63[a]	50.70	55.63[b]	35.70[c]
	3	50.93[d]	47.46[d]	5.93[d]	1.35[d]
	6	45.76[d]	44.46[d]	0[d]	0.40[d]
	9	48.86[d]	44.23d	0[d]	0.43[d]
	SEM	5.14	5.14	5.14	5.14
% MetMb	0	27.60	31.63	23.26[i]	31.63[j]
	3	30.00	29.90	23.93	24.68
	6	24.76	22.43	24.73	24.66
	9	24.03	26.76	23.83[k]	24.86
	SEM	2.67	2.67	2.67	2.67

For [a,b, c, d,e, f, g, h, I, j, k] superscripts, means in the row within each relative pigment concentration not having a common letter are different ($P<0.05$), SEM = standard error of the mean.

Correlation between color parameters and pigment percentage content

Correlation coefficients were calculated to determine if a statistical relationship existed between the various measurements (Table B6 to B9). For AC treatments CIE b* values are highly correlated with OxyMb% ($r = 0.593$, $p = 0.042$). Moreover, for AV treatment there was a significant correlation between parameters L*, a*, b*, h*, and C* with Mb and with OxyMb content. OxyMb ($r= 0.914$, $P<0.001$) had a higher correlation than Mb ($r= -0.838$, $P<0.001$) to chroma for the CV treatment there was a significant correlation between hue with each of the surface pigments concentrations. Dawson et al.,

(1995) demonstrated that an increase in h* is usually followed by the loss of redness and a blending of more yellowness. High OxyMb content is characteristic of a bright red color and high a* values. Correlation data showed a very strong relationship between OxyMb content and a* values for AV treatment ($r= 0.854$, $P<0.001$) which was unexpected since OxyMb % was low, and the use of low OTR kept the pigment in its original form of Mb throughout display time. Finally, CC treatment showed a correlation between L* a* hue* with MetMb content, and a* hue* with Mb content. These findings do not support the belief that using higher CO_2 concentrations (more than 30%) will increase fresh meat storage life, but will accelerate red meat discoloration (Ledward, 1970; Silliker et al., 1977); since in our study CC ground beef package presented a acceptable level of MetMb content after 9 days storage compared with other treatments.

CONCLUSIONS

From the results of this study it would initially appear that there was no difference between CO_2 and air grinding fresh beef. However, high CO_2 MAP is effective in sustaining the "redness" compared to vacuum package. Meat packed under 100% CO_2 environment could regain its "redness" by day 3 to 9 day storage after a phase of pre-mature browning from day 0 to day 3. Additionally, the better color during 9 days in display due to initial lightness (CIE L*) reduction and simultaneous increase of surface redness (CIE a*) combined with lower OTR films maintained a higher surface a* values for high CO_2 MAP than vacuum packaged with low OTR film. The use of 100% CO_2 MAP with conventional grinding resulted in package collapse and CO_2 grinding reduced or prevented package collapse. Thus CO_2 grinding enables the use of 100% CO_2 thus indirectly extending shelf life

The effectiveness of MAP packaging is generally determined by the amount of CO_2 available to dissolve into the meat, for both ensuring bacteriostatic CO_2 availability and preventing package collapse "snug down" effect. In our study, overall CC treatment presented a better package appearance because of the high presence of CO_2 in package headspace during 9 days storage period which can possibly dissolve extensively over time in the meat avoiding package collapse and bacteria growth. In contrast, AC treatment showed more concavity or deflation of the film cover and bacteria growth. In choosing between the 2 treatments without compromising the attractive red color over package appearance, CC treatment fulfilled these requirements to extend the shelf life of

fresh beef by 6 to 9 days at 2±1°C with more rigid package compared to AC fresh beef package.

In general, AC and CC treatments might be a viable alternative to vacuum package and the primary advantage for the use of high CO_2 MAP is its inhibitory effect on the growth of bacteria and to maintain high color redness (a* values), chroma (c* values), and an acceptable level of OxyMb content indicating an increase in color acceptability during 9 days storage period.

The color of fresh beef meat is largely dependant on the relative proportions and distributions of the 3 pigments, OxyMb, MetMb, and Mb. In our study, the characteristic peaks OxyMb in the central part of the spectrum clearly appear from the very first hours of exposure to air for all treatments. The characteristic of MetMb appeared from day 0 to 3, stabilized from day 3 to 6 before it reappeared from day 6 to 9 due to the increasing proportion of oxidized pigment on the meat surface. Meanwhile, if the value of 40% MetMb pointed out by Greene et al., (1971) as a criterion of rejection by consumers, meat from high concentration of CO_2 did not reach this level (<31%) during 9 days that demonstrates no detrimental effect on color shelf life. Consequently, the meat industry can consider the CO_2 grinding in high CO_2 MAP as an alternative to delay meat discoloration that will result in saving the 15% of retail beef discount due to surface discoloration which corresponds to annual revenue losses of $ 1 billion. (Smith et al., 2000)

APPENDICES

Appendix A

Figures

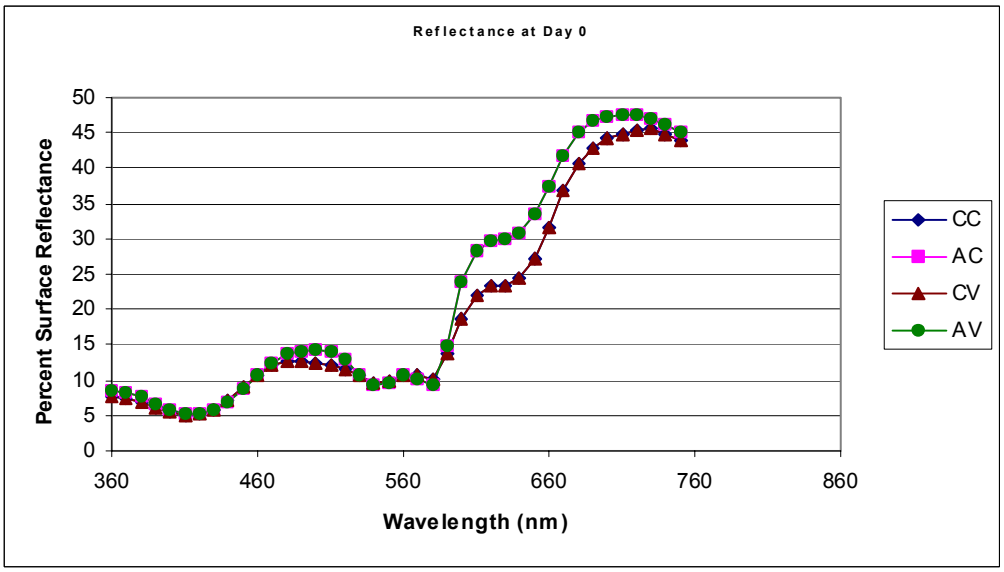

Figure A1: Surface reflectance spectra for all different treatments at day 0.

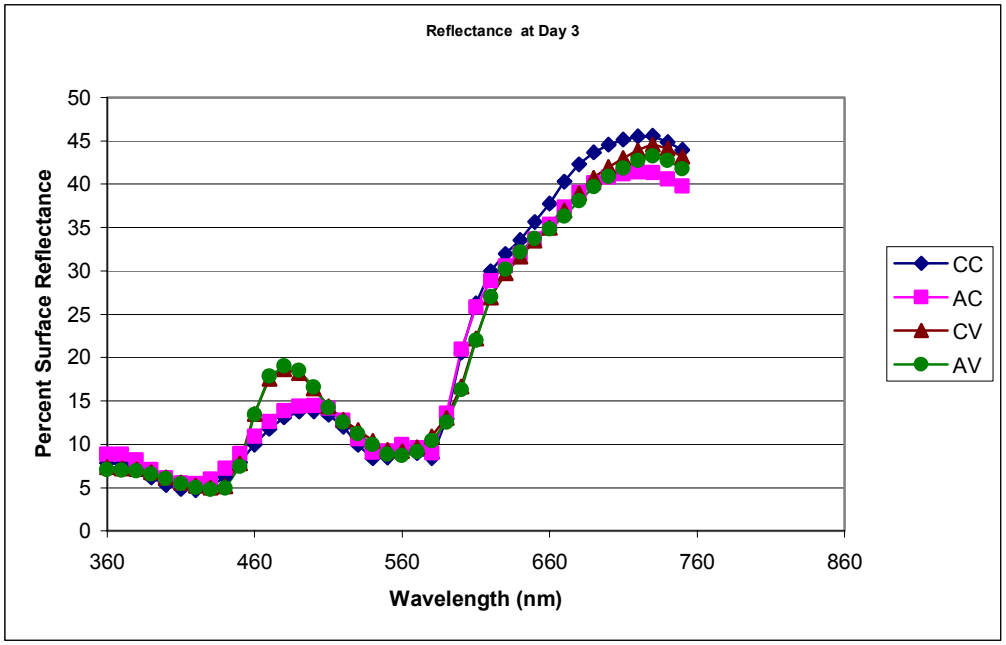

Figure A2: Surface reflectance spectra for all different treatments at day 3.

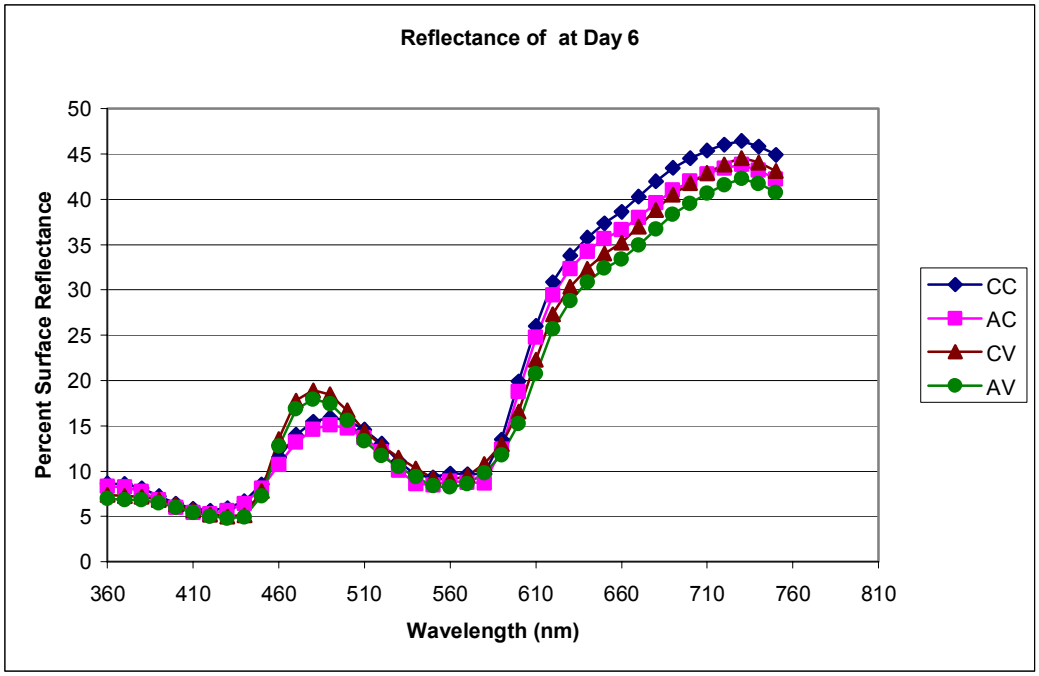

Figure A3: Surface reflectance spectra for all different treatments at day 6.

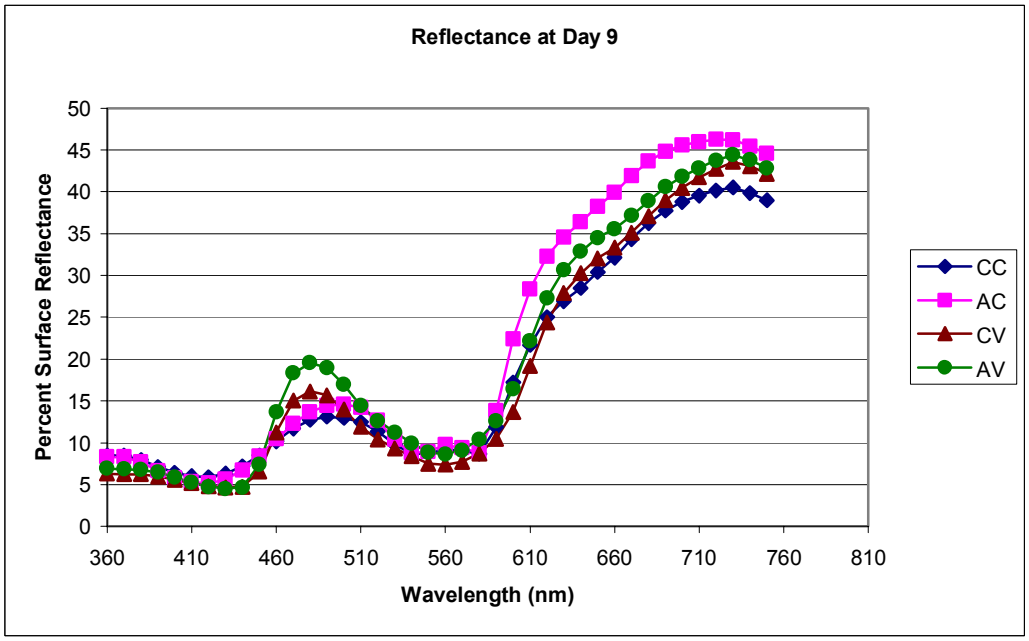

Figure A4: Surface reflectance spectra for all different treatments at day 9.

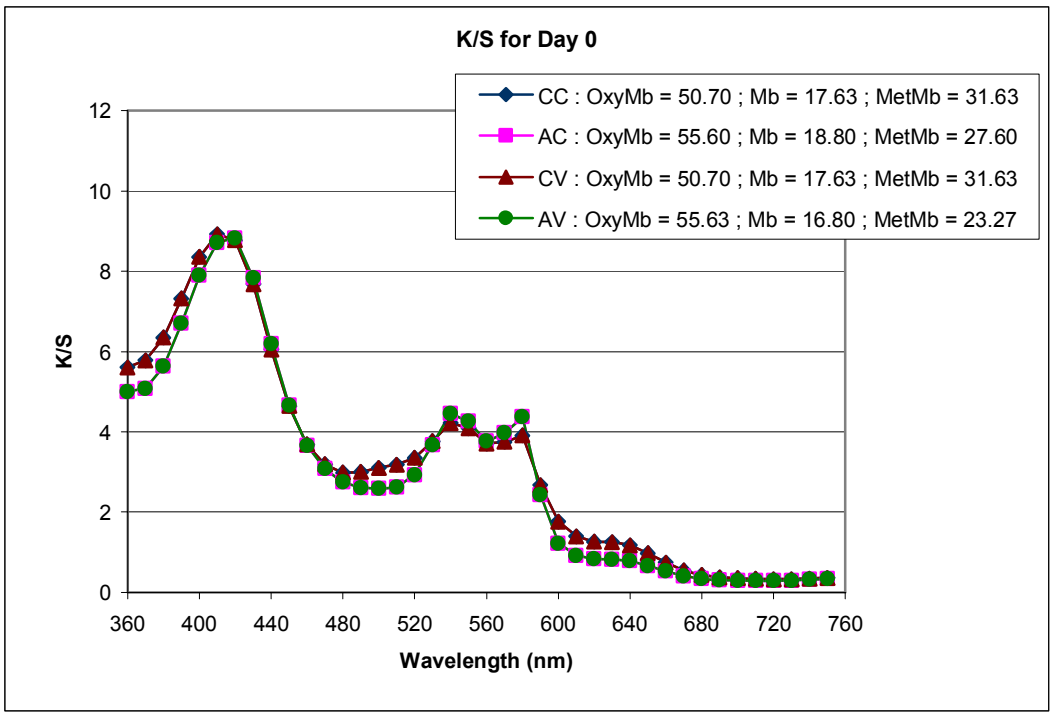

Figure A5: K/S Spectra of Ground Beef for all Different Treatments at Day 0.

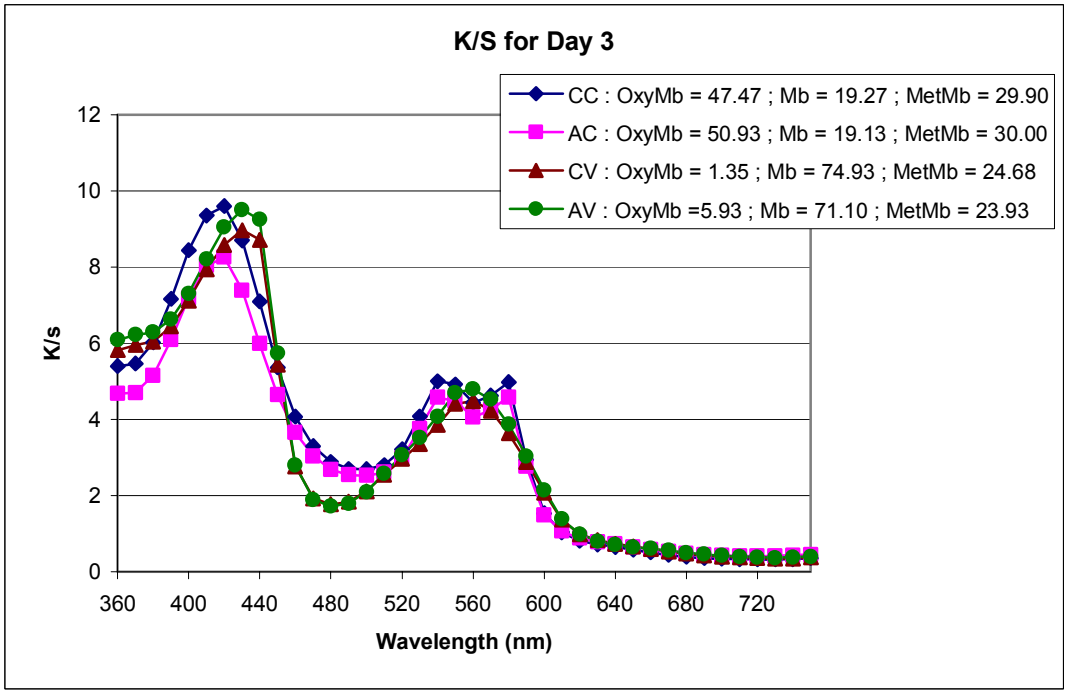

Figure A6: K/S Spectra of Ground Beef for all Different Treatments at Day 3.

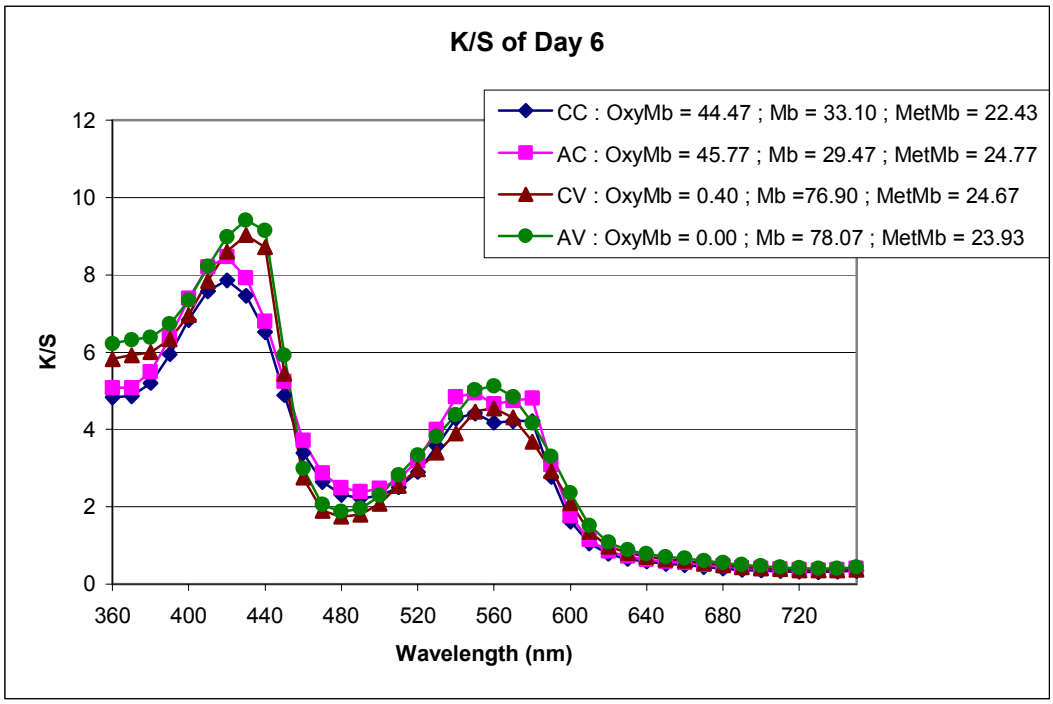

Figure A7: K/S Spectra of Ground Beef for all Different Treatments at Day 6.

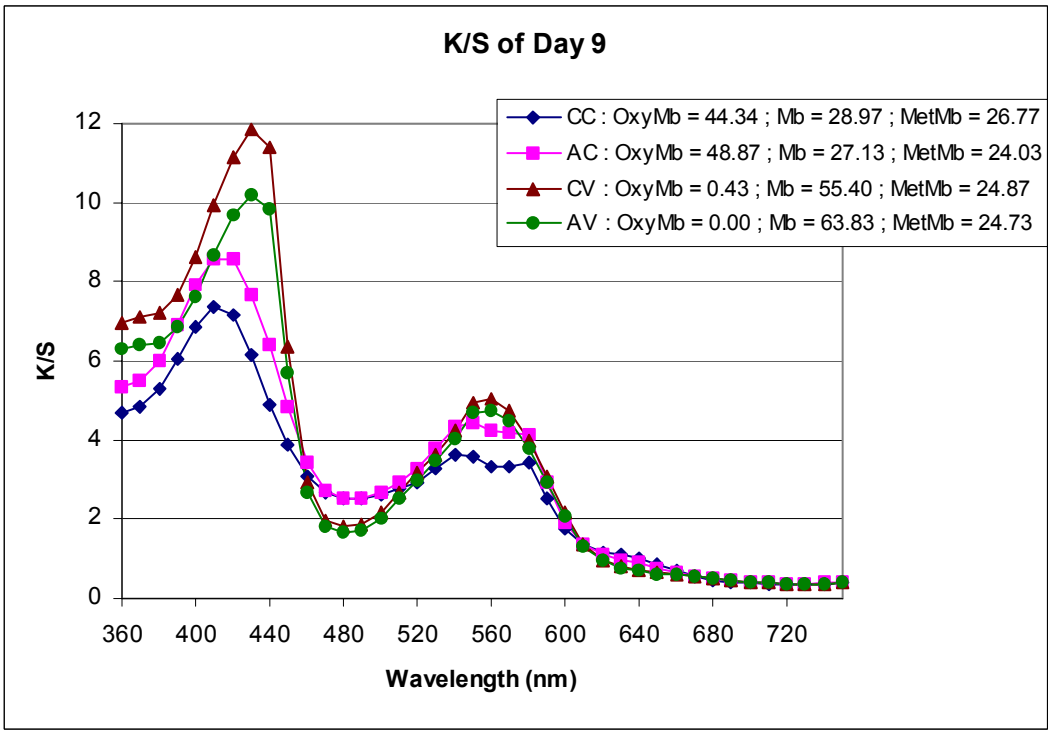

Figure A8: K/S Spectra of Ground Beef for all Different Treatments at Day 9.

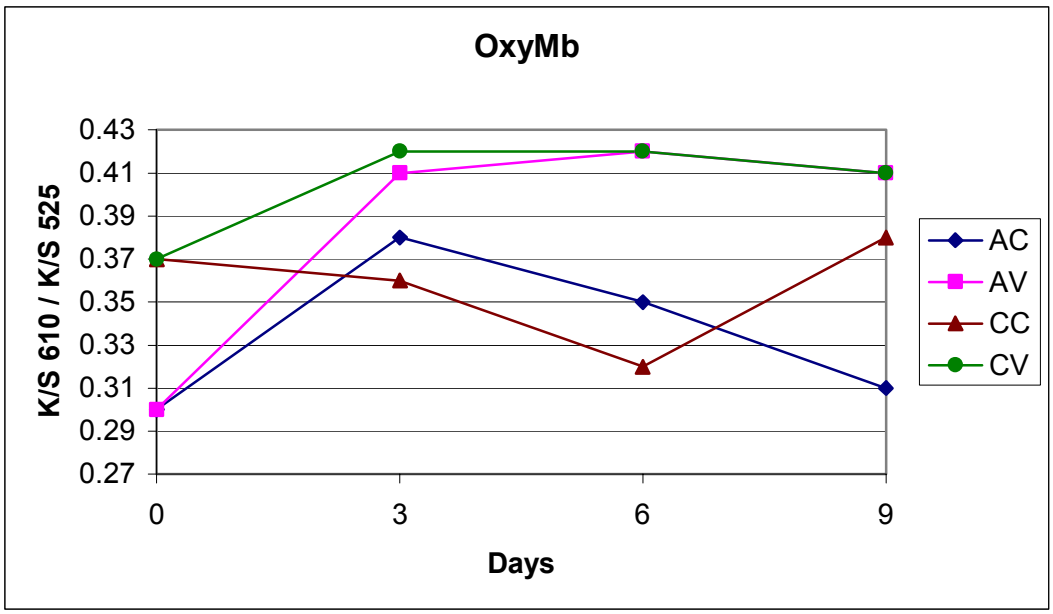

Figure A9: (K/S) 610 / (K/S) 525 Ratio for Surface Oxymyoglobin Formation for Ground beef Meat Displayed in Light for 9 Days.

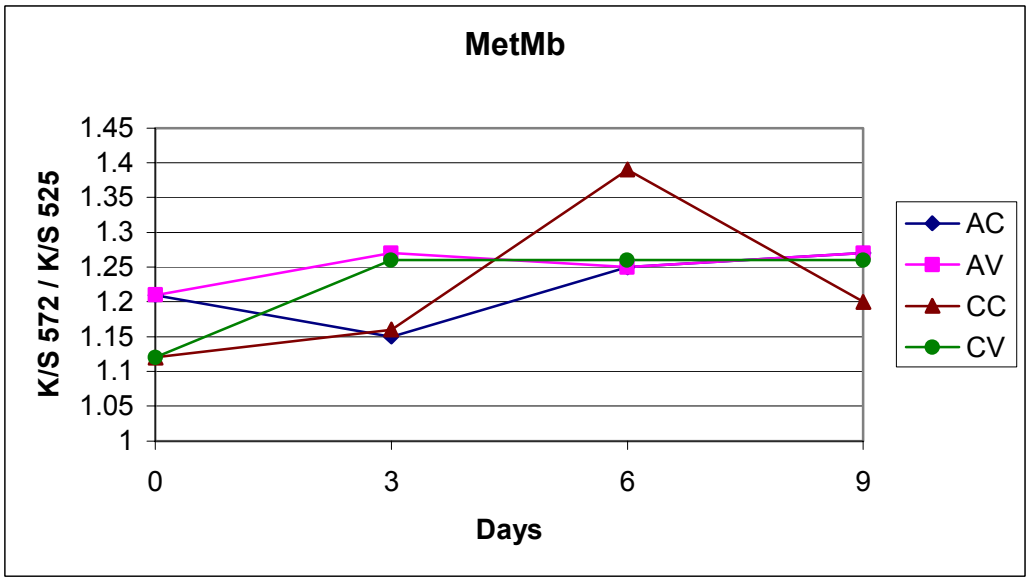

Figure A10: (K/S) 572 / (K/S) 525 Ratio for Surface Metmyoglobin Formation for Ground beef Meat Displayed in Light for 9 Days.

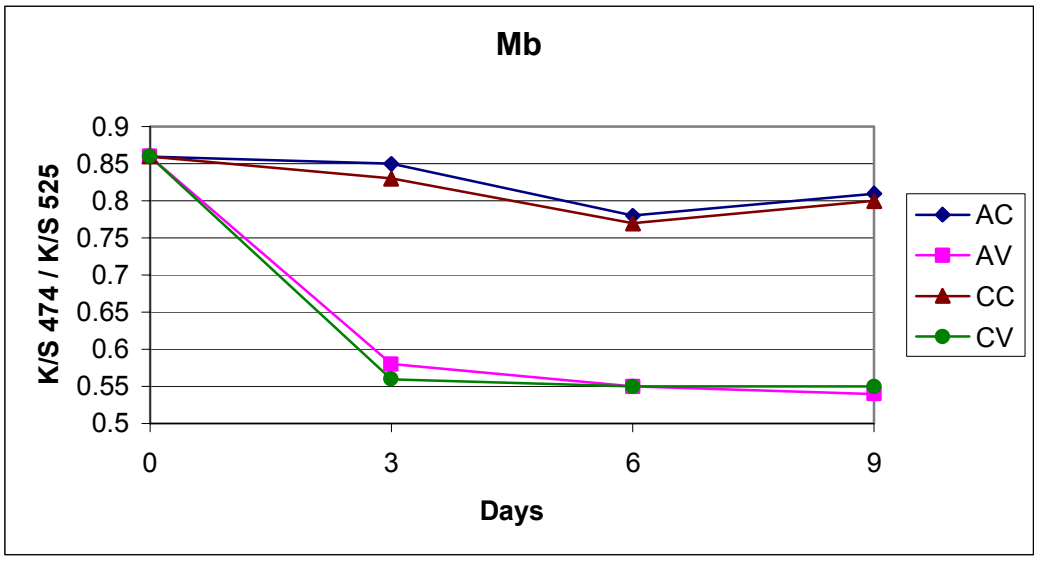

Figure A11: (K/S) 474 / (K/S) 525 Ratio for Surface Myoglobin Formation for Ground beef Meat Displayed in Light for 9 Days.

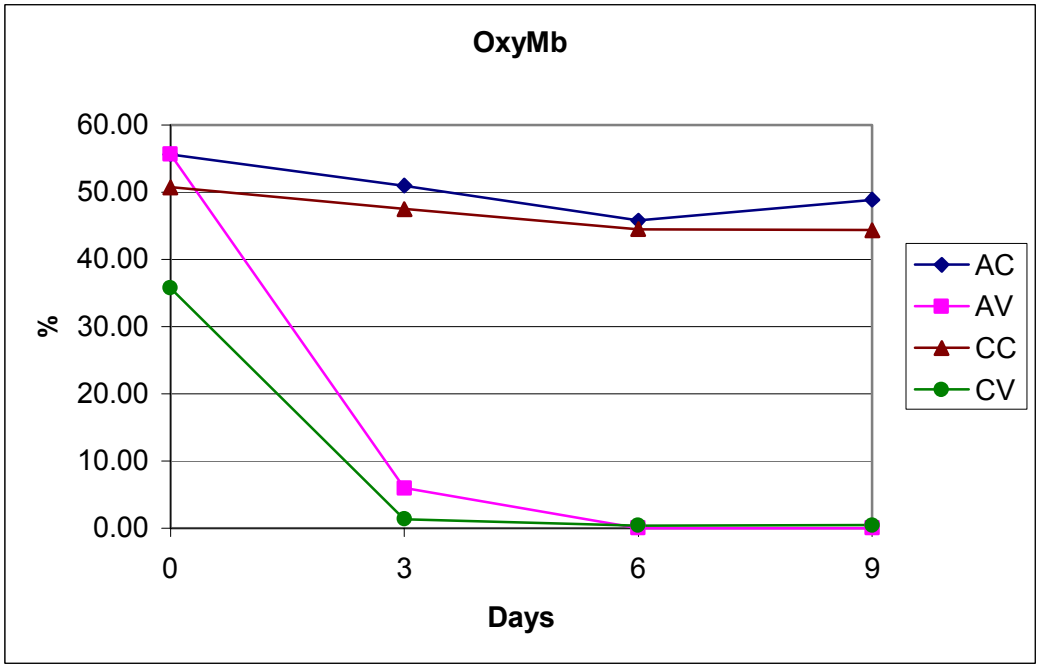

Figure A12: Percentage Surface Oxymyoglobin Formation for Ground beef Meat Displayed in Light for 9 Days.

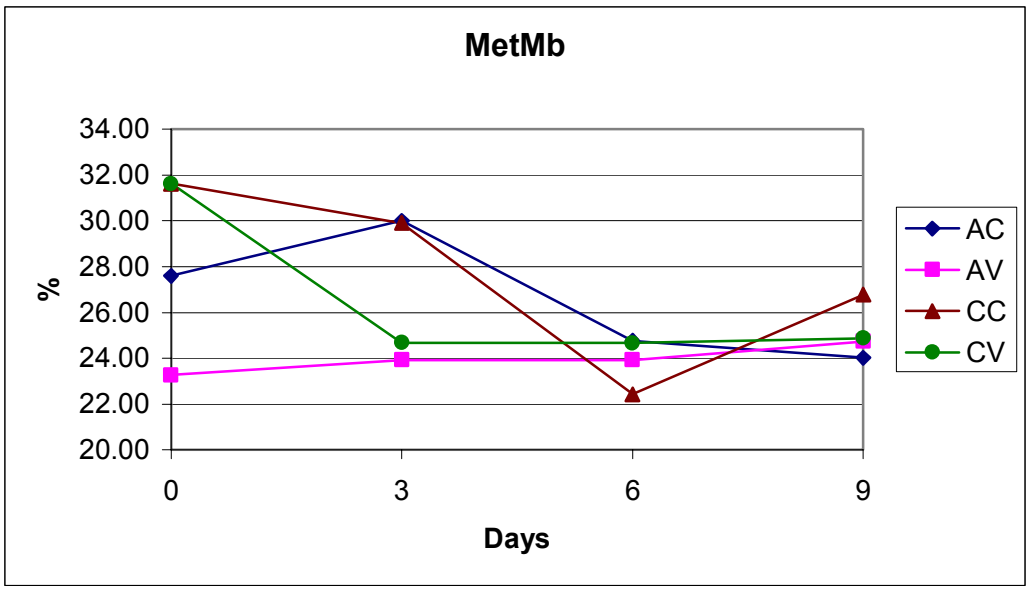

Figure A13: Percentage Surface Metmyoglobin Formation for Ground beef Meat Displayed in Light for 9 Days.

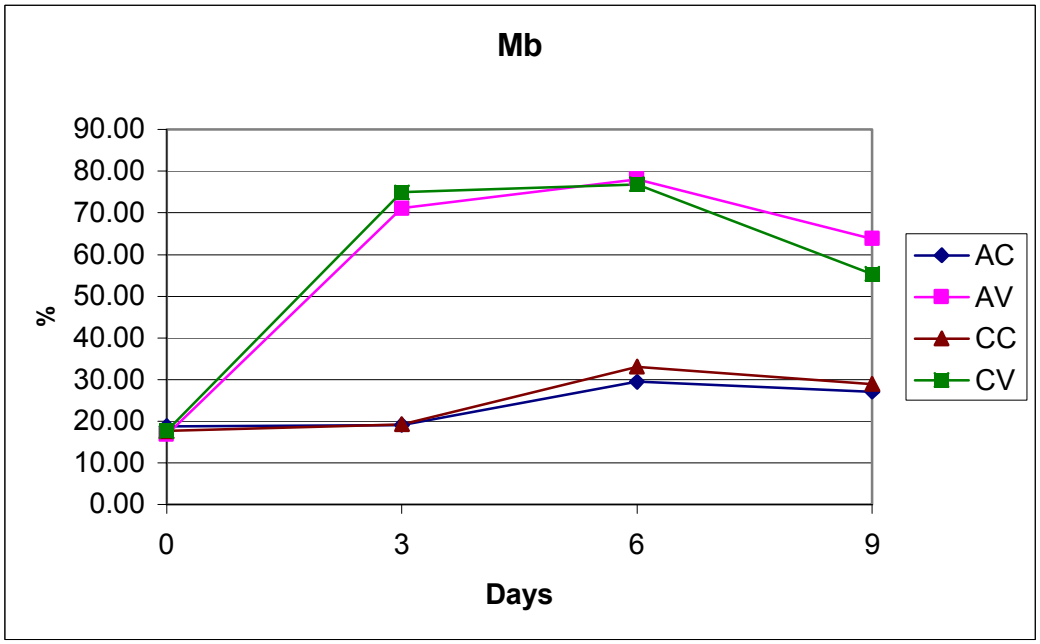

Figure A14: Percentage Surface Myoglobin Formation for Ground beef Meat Displayed in Light for 9 Days

Appendix B

Tables

Table B1: Mean total plate count for all the treatments throughout the storage period.

Time (Days)	AC (LogCFU/g)	CC (LogCFU/g)	AV (LogCFU/g)	CV (LogCFU/g)
0	4.91^{a}	4.40^{b}	4.91^{a}	4.40^{b}
3	$5.13^{a\,e}$	4.90^{a}	5.62^{c}	$5.54^{c\,e}$
6	5.97^{b}	5.61^{c}	$6.74^{d\,g}$	$6.73^{f\,g}$
9	$6.35^{b\,g}$	$6.25^{d\,g}$	$7.38^{e\,h}$	$7.44^{a\,h}$

$*^{abcdefgh}$ Means without a common superscripts in each row are significantly ($P<0.05$) different.

abcdefgh Means without a common superscripts in each column are significantly ($P<0.05$) different
AC: Air grinding/ 100 % CO_2 MAP
CC: CO_2 grinding/ 100% CO_2 MAP
AV: Air grinding/ Vacuum package
CV: CO_2 grinding/ Vacuum package
Standard error of mean (SEM) = 0.188

Table B2: Change in mean CO_2, O_2 and N_2 concentration for the empty container throughout the storage period.

Time (Days)	CO_2 (%)	O_2 (%)	N_2 (%)
0	96.41	0.28	3.29
3	95.41	0.40	4.18
6	95.81	0.34	3.62
9	94.94	0.61	4.30
SEM	1.032	0.264	0.833

Table B3: Least square means for (K/S)610/(K/S)525 values For all treatments over 9 day period storage.

Treatment	Display day			
	0	3	6	9
AC	0.303	0.380	0.350	0.310
CC	0.366	0.356	0.323	0.380
AV	0.303	0.410	0.423	0.413
CV	0.366	0.423	0.416	0.406

SEM is the standard error of the least squares means = 0.021

Table B4: Least square means for (K/S)474/(K/S)525 values For all treatments over 9 day period storage.

Treatment	Display day			
	0	3	6	9
AC	0.863	0.853	0.780	0.806
CC	0.863	0.830	0.766	0.800
AV	0.863	0.576	0.550	0.536
CV	0.863	0.563	0.550	0.546

SEM is the standard error of the least squares means = 0.017

Table B5: Least square means for (K/S)572/(K/S)525 values for all treatments over 9 day period storage.

Treatment	Display day			
	0	3	6	9
AC	1.206	1.146	1.250	1.270
CC	1.120	1.160	1.296	1.203
AV	1.206	1.266	1.250	1.270
CV	1.120	1.256	1.256	1.260

SEM is the standard error of the least squares means = 0.043

Table B6: Correlation (r) and p-value (P) between percent surface pigment and L*a*b*h* and C* for AC TRT

Pigment (%)	L	a	b	Hue	Chroma
MetMb(r)	0.33	-0.55	-0.12	0.35	-0.47
MetMb(P)	0.28	0.05	0.69	0.25	0.11
Mb(r)	-0.34	-0.18	-0.47	-0.51	-0.26
Mb(P)	0.27	0.56	0.11	0.08	0.40
OxyMb(r)	0.16	0.53	0.59	0.34	0.57
OxyMb(P)	0.59	0.07	0.04	0.27	0.05

Table B7: Correlation (r) and p-value (P) between percent surface pigment and L*a*b*h* and C* for AV TRT

Pigment (%)	L	a	b	Hue	Chroma
MetMb(r)	-0.02	0.20	-0.003	0.06	0.12
MetMb(P)	0.94	0.52	0.99	0.85	0.69
Mb(r)	-0.65	-0.79	-0.84	-0.85	-0.83
Mb(P)	0.02	0.001	0.0005	0.0004	0.0006
OxyMb(r)	0.73	0.85	0.95	0.96	0.91
OxyMb(P)	0.006	0.0004	<0.0001	<0.0001	<0.0001

Table B8: Correlation (r) and p-value (P) between percent surface pigment and L*a*b*h* and C* for CC TRT

Pigment (%)	L	a	b	Hue	Chroma
MetMb(r)	0.59	-0.71	0.46	0.70	-0.39
MetMb(P)	0.04	0.0096	0.12	0.01	0.19
Mb(r)	-0.57	0.65	-0.40	-0.60	0.38
Mb(P)	0.05	0.02	0.18	0.03	0.21
OxyMb(r)	-0.02	-0.10	0.16	0.16	-0.02
OxyMb(P)	0.94	0.74	0.60	0.60	0.94

Table B9: Correlation (r) and p-value (P) between percent surface pigment and L*a*b*h* and C* for CV TRT

Pigment (%)	L	a	b	Hue	Chroma
MetMb(r)	0.55	-0.11	0.84	0.87	0.32
MetMb(P)	0.05	0.58	0.0005	0.0002	0.30
Mb(r)	-0.36	-0.11	-0.74	-0.74	-0.49
Mb(P)	0.24	0.71	0.005	0.005	0.09
OxyMb(r)	0.57	-0.42	0.66	0.74	0.02
OxyMb(P)	0.04	0.14	0.01	0.005	0.95

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