EFFECTS OF PACKAGING ATMOSPHERES AND INJECTION ENHANCEMENT ON
BEEF POSTMORTEM PROTEOLYSIS, INSTRUMENTAL TENDERNESS, SENSORY
TRAITS, AND DISPLAY COLOR

by

JEANNINE PATRICIA GROBBEL

B.S., Michigan State University, 2002
M.S., Kansas State University, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Food Science Institute
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Abstract

The objectives were to determine the effects of packaging and injection-enhancement on beef sensory attributes, postmortem proteolysis, and color. Muscles from USDA Select, A-maturity carcasses were fabricated into 2.54-cm steaks on d 7 postmortem. In Experiment 1, longissimus lumborum (n=14 pairs) muscles were used. Packaging treatments were: vacuum packaging (VP); 80% O_2/20% CO_2 (HiO$_2$); 0.4% CO/35% CO_2/64.6%N_2 (ULO$_2$CO); 0.4% CO/99.6% CO_2; 0.4% CO/99.6% N_2; or 0.4% CO/99.6% Ar. In Experiment 2, longissimus lumborum (n=12 pairs); semitendinosus (n=12 pairs); and triceps brachii (n=24 pairs) muscles from one carcass side were injection-enhanced or non-enhanced. Steaks were packaged into VP, HiO$_2$, or ULO$_2$CO MAP. Steaks packaged in HiO$_2$ MAP were in dark storage (2°C) for 4 d and all other steaks for 14 d. Steaks were displayed under fluorescent lighting for 7 d. Trained color panelists assigned color scores. Steaks for tenderness, cooked color, and sensory were cooked to 70°C. Steaks packaged in VP or ULO$_2$ with CO MAP had little or no discoloration. Steaks packaged in HiO$_2$ MAP discolored faster ($P < 0.05$) and more ($P < 0.05$) than steaks in other packaging treatments. Steaks packaged in HiO$_2$ MAP were less tender ($P < 0.05$) than other treatments at the end of display, but had 10 d less aging due to shorter dark storage. Steaks packaged in HiO$_2$ had the lowest ($P < 0.05$) a* values for internal cooked color of all treatments and exhibited premature browning. Enhanced steaks were more tender ($P < 0.05$) than non-enhanced steaks. Sensory panelists found that non-enhanced steaks packaged in ULO$_2$CO MAP or VP were more tender ($P < 0.05$), had more ($P < 0.05$) beef flavor, and had less ($P < 0.05$) off-flavors than steaks packaged in HiO$_2$ MAP. Off-flavors for steaks packaged in HiO$_2$ MAP often were described as oxidative and rancid. Enhanced steaks had more ($P < 0.05$) off-flavors than non-enhanced steaks. Postmortem proteolysis measured by desmin degradation was not affected ($P > 0.05$) by packaging. Steaks packaged in ULO$_2$ plus CO MAP had superior color stability, tenderness, and sensory attributes compared to steaks in HiO$_2$ MAP.

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Dedication

I dedicate my dissertation to my parents, Jerry and Lynne, and grandparents, Jean Patricia and Jim, for all of their love, support, and prayers.

CHAPTER 1 - Literature Review

Many meat quality traits are involved in consumers' overall purchase decisions and satisfaction of meat products. Color is the major factor affecting consumers' purchasing decisions, while tenderness, juiciness, and flavor contribute to the overall eating satisfaction and palatability. In recent years, case-ready meat has become a larger share of the marketplace. The type of packaging used and the different atmospheres associated with case-ready meat can alter the color of meat in addition to sensory attributes.

PACKAGING

Packaging is the material used to enclose a product and for meat can consist of bags, pouches, trays, wraps, or a combination of these (Kropf, 2004). There are certain inherent functions or properties of packaging that directly impact the characteristics of the product packaged and in the case of meat, color is greatly impacted. Kropf (2004) describes protection from environmental variables as a major function of packaging. In addition, barrier properties and permeability of oxygen and water vapor of the package materials will have a huge influence on the packaged product.

Meat that is fabricated from vacuum packaged subprimals and packaged for retail display and sale is traditionally done so on polystyrene foam trays (Cole, 1986) with highly-oxygen permeable, polyvinyl-chloride-overwrap film (PVC). In recent years, however, modified atmosphere packaging (MAP) has increased in popularity and use. Currently, MAP is the primary packaging used for case-ready meats, whereby cuts are produced at a central location and packaged in MAP. Packages with high oxygen (HiO$_2$), low oxygen, or ultra-low oxygen (ULO$_2$) are considered MAP (Kropf, 2000).

MODIFIED ATMOSPHERE PACKAGING

Eilert (2005) reported that case-ready packaging in the meat industry was growing at a rapid rate citing an industry study by Mize and Kelly (2004) indicating that 60% of packages in the retail case were in the case-ready format as opposed to only 49% in 2002. Mize and Kelly (2004) reported only 23% of beef was in the case ready format, with 66% of ground beef

packages being case ready, whereas 95% of poultry was packaged as case ready. It is important to note that the Mize and Kelly (2004) study looked at package numbers and not total volume of meat. It is assumed that, currently, case-ready fresh beef at the retail level is enhanced and displayed in modified atmosphere packaging (MAP), with the majority being in HiO_2 (80% O_2/ 20% CO_2) atmosphere. Eilert (2005) predicted the use of ultra-low oxygen MAP with CO will increase, especially with increased distribution areas obtained with this technology by increasing the shelf-life and allowing packages to be shipped farther from the centralized packaging facility. Case-ready meat provides many benefits to meat processors due to centralized packaging, including better microbial control, extended shelf life, and quality control. In addition, the need for skilled labor capable of fabricating meat in retail stores is no longer needed with centralized packaging (Eilert, 2005).

There are several advantages of using MAP, besides packaging at a central location. These include less handling, improved control of sanitation, more consistent products, increased marketing flexibility, and the ability to maintain more accurate records of inventory (Cole, 1986; Jeyamkóndan et al., 2000; Kropf, 2004). Additionally, when CO_2 is present in packages, it inhibits microbial growth (Clark and Lentz, 1969; Baran et al., 1970; Sørheim et al., 1999). Nitrogen is also used to prevent package collapse when dissolution of CO_2 takes place (Cole, 1986; Kropf, 2000). For MAP to be effective, low temperature storage is critical (Jeyamkóndan et al., 2000).

Along with the advantages of using MAP, there come some detrimental quality issues and disadvantages. Meat packaged in MAP has increased package cost and has been associated with off flavors and/or odors (Kropf, 2004).

EFFECTS OF PACKAGING ON TENDERNESS AND SENSORY TRAITS

In order to provide consumers with consistent and desirable products, it is necessary to fully understand the effects that packaging atmospheres have on important beef quality traits, including palatability factors of tenderness, juiciness, and flavor. Providing consistent, high quality beef to consumers is of utmost importance in order to maintain customer satisfaction and allow for repeat purchases.

Tenderness is the most important palatability attribute (Dikeman, 1987; Miller et al., 1995); however, there is huge variation in tenderness among different muscles and cuts.

Tenderness problems exist in strip loin steaks purchased at the retail level. George et al. (1999) reported that 24% of strip loin steaks purchased from across the nation at the retail level were classified as "slightly tough" or tougher by a trained sensory panel. Additionally, consumers have the ability to determine tenderness and many are willing to pay a premium for "guaranteed tender" steaks (Mintert et al., 2000; Miller et al., 2001).

Packaging meat in HiO_2 MAP results in a desirable bright red display color (Behrends et al., 2003; Seyfert et al., 2005; Sekar et al., 2006) but may have detrimental effects on other quality characteristics, including increased off-flavors and decreased tenderness, as well as bone discoloration (Grobbel et al., 2006). Tørngren (2003) found that steaks aged and packaged in HiO_2 MAP had more off-odor and off-flavor, including warmed-over flavor, with increased duration of storage in HiO_2 MAP. Steaks aged in HiO_2 MAP also were less tender and juicy and had more premature browning than steaks aged in vacuum packaging and then packaged in PVC overwrap. Sørheim et al. (2004) found that steaks packaged in HiO_2 MAP were less tender than vacuum packaged steaks according to both instrumental and sensory tests. They also found that steaks packaged in HiO_2 MAP had more rancid taste and were less juicy than steaks vacuum packaged.

Clausen (2004) found that beef loin steaks aged in 100% nitrogen (N_2) were equally as tender and juicy as those aged as a whole loin in vacuum packaging but more tender than those aged in vacuum skin packaging as steaks or those steaks packaged in 50% O_2/50% CO_2. They also found steaks packaged or aged in HiO_2 MAP to be less juicy and have more warmed over flavor than steaks packaged and displayed in air or vacuum skin packaging.

Madsen and Clausen (2006) evaluated beef loin steaks packaged in HiO_2 MAP, ULO_2 MAP with and without carbon monoxide (CO), and vacuum packaging. They reported that steaks packaged in HiO_2 MAP were less tender, had more warmed-over flavor, and less meat flavor than steaks packaged in vacuum skin packaging and ULO_2 with and without CO. They also found that steaks packaged in HiO_2 MAP had lower cook loss than steaks packaged in MAP with high levels of CO_2 (60%) with or without 0.4% CO.

Although only small numbers of animals were used, Tørngren (2003) conducted a study that utilized cows and heifers, whereas Sørheim et al. (2004) used bulls, and Clausen (2004) used cows and heifers to consistently show declines in tenderness due to O_2 in the packages. Because differences in tenderness and color exist among intact and non-intact males and among animals

of different ages, it is ideal to investigate the effects of different packaging systems on animals more representative of the U.S. beef industry.

Jackson et al. (1992) evaluated the volatile compounds from the headspace of beef strip loins vacuum packaged or in: 100% CO_2 MAP; 40% CO_2/ 60% N_2 MAP; or HiO_2 (80% O_2/ 20% CO_2) MAP and evaluated microbial changes. Steaks packaged in HiO_2 MAP developed strong off-odors and had methyl thiirane, ethyl acetate, benzene and 1-heptene in the packages after 7 and 14 d of storage but not in the vacuum packaged steaks or other MAP atmospheres. They reported the highest aerobic plate counts for steaks stored in HiO_2 MAP and the lowest counts for steaks stored in 100% CO_2 MAP and vacuum packaging. Strip loins stored in HiO_2 MAP were predominantly contaminated with *Pseudomonas putida*, while *Lactobacillus plantarum* were the major microbes in vacuum packaged strip loins, and *Leuconostoc mesenteroides* were found in loins stored in 100% CO_2 MAP.

Sekar et al. (2006) looked at buffalo quadriceps packaged in HiO_2 MAP, vacuum, or aerobically and found that vacuum packaged meat had a smaller fiber diameters, longer sarcomere lengths, and lower myofibrillar fragmentation indexes overall for 21 d of storage than steaks packaged in HiO_2 MAP. They attribute these changes to the aging process and postmortem proteolysis and believe that the reducing environment associated with vacuum packaging allowed these changes to occur while HiO_2 MAP was not capable of resulting in similar changes in muscle structure postmortem. They reported that steaks packaged in HiO_2 MAP had better color stability than buffalo steaks packaged in vacuum or aerobically.

Lee et al. (1996) compared electron-beam irradiation (2 kGy) of prerigor beef longissimus steaks packaged in ULO_2 MAP (75% N_2/ 25% CO_2) and stored at 15°C or 20°C for 3 d to postrigor vacuum packaged longissimus steaks stored at 2° for 14 d. They reported similar WBSF values for irradiated samples stored at 30°C for 2 d as traditionally vacuum aged steaks at 14 d postmortem. They also found reduced thiobarbituric acid reactive substances (TBARS) values with irradiated steaks and no detrimental impact on microbial growth by storing irradiated beef at higher temperatures. They concluded that combining irradiation of prerigor steaks with MAP packaging at higher temperatures for 2 d and then storing steaks in MAP at refrigeration temperatures would result in an accelerated method for safely aging beef steaks.

Beggan et al. (2004) packaged beef longissimus, semimembranosus, psoas major, and gluteus medius muscles in low oxygen and vacuum packages for 3 wk dark storage and then

either perforated the packages or repackaged in PVC overwrap for display. Overall, they found no differences in shear force or microbiology among display treatments. They evaluated sensory attributes and found minimal differences in juiciness and beef flavor. They reported that psoas major steaks packaged in the intact low-oxygen package were moister than steaks stored in vacuum and then cut and overwrapped for display. They also reported that semimembranosus steaks in the intact, low-oxygen packages to have better flavor than overwrapped steaks.

Smulders et al. (2006) looked at storing beef loins in large stainless steel containers at subatmospheric pressure (25 kPa) with modified atmospheres of 100% CO_2 or 70% N_2 and 30% CO_2 to storing beef loins in vacuum packaging. They found no differences in shear force due to packaging method at subatmospheric pressure and only slight changes in color, but they reported increased purge loss with the modified atmosphere storage than vacuum packaging.

Campo et al. (2006) compared TBARS and sensory analysis of beef with different fatty acid profiles from several different feeding trials that was stored in HiO_2 MAP (75% O_2/ 25% CO_2) for 0, 4, or 9 d in simulated retail display. They found no oxidation on d 0 of display but there was an increase in TBARS from d 0 to d 4 and then a greater increase from d 4 to d 9 of display with variation in the amount of oxidation. They reported high correlations of TBARS with beef flavor (-0.80), abnormal (0.82), rancid (0.84) and greasy (0.70) flavors. According to sensory panelists, as abnormal or rancid flavor increased in the samples, beef flavor intensity decreased as display time increased. They reported a TBARS value of approximately 2 to potentially be the threshold for acceptability of oxidized beef under their experimental conditions.

Chevon longissimus, semimembranosus, and triceps brachii steaks were packaged in vacuum packaging or PVC overwrap 24 h postmortem and stored for 0, 4, 8, or 12 d (Kannan et al., 2002). They reported no differences in Warner-Bratzler shear force due to packaging method but did find differences among muscles.

Packaging alters tenderness and sensory traits. In general, steaks packaged in HiO_2 MAP have more negative effects than steaks packaged in other packaging types. More research is needed to evaluate the effects of packaging on U.S. grain-fed beef tenderness and sensory attributes.

POSTMORTEM PROTEOLYSIS AND PROTEIN OXIDATION

Aging of meat increases tenderness and is mostly explained through the increase in proteolysis that occurs during aging when key proteins, desmin and titin, are broken down (Koohmaraie, 1994; Koohmaraie and Geesink, 2006). In an excellent review paper, Koohmaraie and Geesink (2006) reported that "meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, and postmortem proteolysis of myofibrillar and myofibrillar-associated proteins." The calpain system is likely responsible for the majority of postmortem proteolysis (Koohmaraie, 1994), with μ-calpain being the calcium-activated protease almost entirely responsible for postmortem tenderization (Koohmaraie and Geesink, 2006).

In a study looking at the effects of early postmortem protein oxidation on beef quality, Rowe et al. (2004a) evaluated beef loin steaks from animals fed vitamin E supplementation or no vitamin E supplementation that were either irradiated or non-irradiated within 26 h postmortem. They reported detrimental effects on fresh beef color due to irradiation and found that vitamin E supplementation did not protect irradiated steaks from becoming darker and less red. They also reported that irradiated steaks had less protein solubility at 3 d post irradiation than non-irradiated steaks. They found total carbonyl content (an indication of protein oxidation) to be higher in sarcoplasmic and myofibrillar proteins on d 0, 1, 3, and 7 in irradiated steaks than in non-irradiated steaks, and higher in myofibrillar proteins after 14 d of irradiation as well. According to Western blots, they also showed irradiated steaks had more oxidized sarcoplasmic and myofibrillar proteins than non-irradiated steaks, but vitamin E prevented some sarcoplasmic protein oxidation. They reported carbonyl content 1 d after irradiation to be correlated with 14 d WBSF values, thus implying that protein oxidation early postmortem may be associated with decreased beef tenderness even after 14 d of aging.

Both Clausen (2004) and Sørheim et al. (2004) attribute the reduction in tenderness of steaks in HiO_2 MAP to be caused by the O_2 and not by CO_2 or N_2. Clausen (2004) believes that the detrimental effects of O_2 on tenderness could be caused by protein oxidation. Adding to what Rowe et al. (2004a) reported, Rowe et al. (2004b) found that oxidation of beef steak proteins early postmortem inactivated μ-calpain and decreased myofibrillar proteolysis and thus limited the extent of tenderization. Therefore, the inclusion of O_2 at high levels, such as what is found in HiO_2 MAP, may be limiting postmortem proteolysis and not allowing steaks packaged

in such environments to tenderize to their fullest potential. It appears that ULO_2 MAP atmospheres do not have negative effects on meat tenderness, but it is important to evaluate these environments to determine if one or more may have beneficial effects on tenderness.

FRESH MEAT COLOR STABILITY

There are several factors that contribute to a muscle's color stability and ability to maintain good color in the display case. Mancini and Hunt (2005) reviewed many of these ante- and post-mortem factors that contribute to muscle color. Conversion of pigment forms through oxygenation, oxidation, oxidation plus reduction, or the formation of carboxymyoglobin of a muscle is associated with the color of meat and its stability (Figure 1.1). Factors affecting the pigment forms or formation of certain pigments are numerous. In addition, differences in color can be linked to a muscle's metmyoglobin reducing activity (Seyfert et al., 2006). McKenna et al. (2005) characterized 19 different bovine muscles and, in general, muscles with high color stability were the longissimus, semitendinosus, and tensor fasciae latae. These muscles were associated with high resistance to induced metmyoglobin formation, nitric oxide reducing ability, and oxygen penetration depth, whereas they typically had low oxygen consumption rates, myoglobin content, and oxidative rancidity. They also reported that muscles associated with low color stability were the triceps brachii, serratus ventralis, supraspinatus, infraspinatus, and psoas major. These muscles had high metmyoglobin reductase activity, oxygen consumption rates, myoglobin content, and oxidative rancidity while being low in resistance to induced metmyoglobin formation, nitric oxide reducing ability, and oxygen penetration depth.

High-oxygen MAP has been shown to maintain a desirable bright, cherry-red color with extended display compared to steaks packaged in PVC. Tørngren (2003) showed that beef loin steaks aged in vacuum packages and then displayed in PVC overwrap or HiO_2 MAP had higher a* values (redder) than steaks aged and displayed in MAP. Beef steaks packaged in 80% O_2 maintained a desirable red color for 14 d of storage and steaks packaged in ULO_2 plus 0.4% CO had desirable red color for 21 d of storage (John et al., 2005).

Seyfert et al. (2004a) compared color of injection-enhanced beef from hot- or cold-boned quadriceps muscles packaged in HiO_2 MAP or ULO_2 MAP. Steaks packaged in HiO_2 MAP were stored in dark storage until 12 d postmortem and steaks packaged in ULO_2 MAP were stored for 21 d postmortem at which point they were displayed for 5 d (HiO_2 MAP) or 3 d (ULO_2

MAP). Packages of ULO_2 MAP had a barrier lidding film removed at the time they were put into display, allowing steaks to bloom to a cherry red color. They found that steaks packaged in HiO_2 MAP were brighter, more cherry-red and had more color stability than steaks packaged in ULO_2 MAP, even with increased display times for steaks in HiO_2 MAP.

Figure 1.1 Visible myoglobin redox interconversions on the surface of meat. Based in part on Brooks (1935), Livingston and Brown (1981), and Wallace et al. (1982). From Mancini and Hunt, 2005.

R.A. Mancini, M.C. Hunt / Meat Science 71 (2005) 100–121

Rx 1 (Oxygenation): $DMb + O_2 \rightarrow OMb$

Rx 2a (Oxidation): $OMb + [\text{oxygen consumption or low } O_2 \text{ partial pressure}] - e^- \rightarrow MMb$

Rx 2b (Oxidation): $[\text{DMb} - \text{hydroxyl ion} - \text{Hydrogen ion complex}] + O_2 \rightarrow MMb + O_2^-$

Rx 3 (Reduction): $MMb + \text{Oxygen consumption} + \text{metmyoglobin reducing activty} \rightarrow DMb$

Rx 4 (CarboxyMb): $DMb + \text{carbon monoxide} \rightarrow COMb$
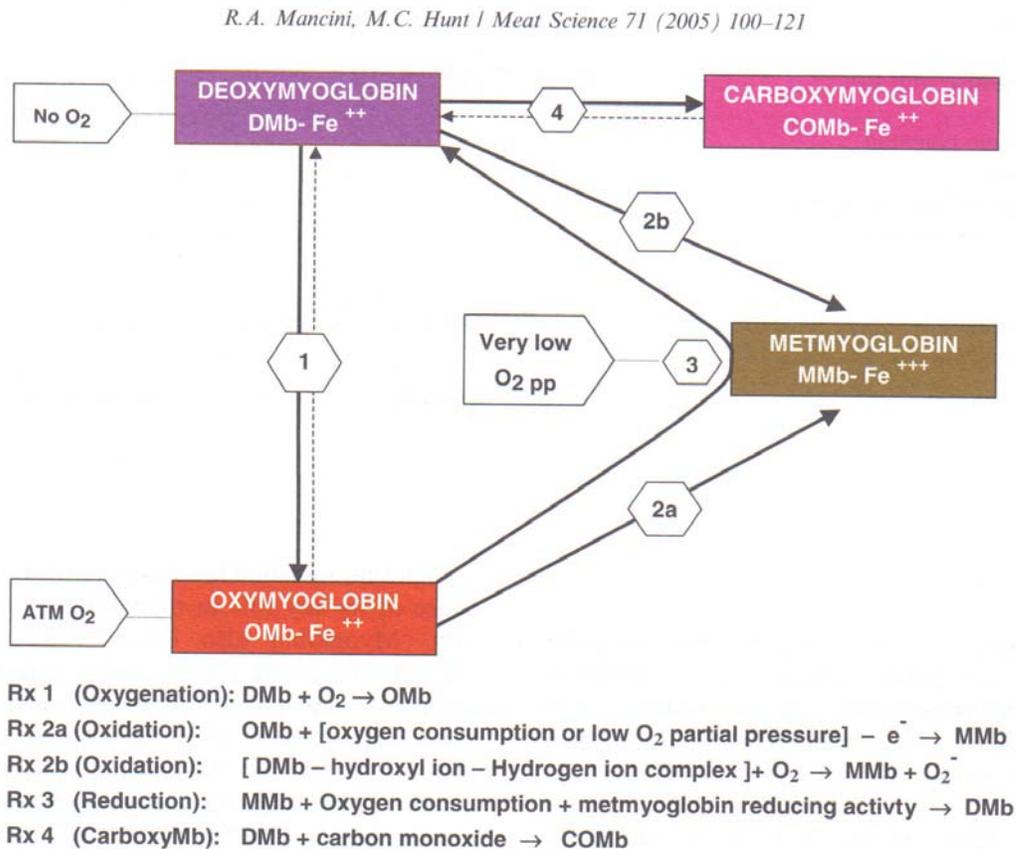
Comparisons among USDA Select, low Choice, and high Choice beef semimembranosus, semitendinosus, and biceps femoris steaks packaged in HiO_2 MAP or PVC were made for color and discoloration (metmyoglobin formation) (Behrends et al., 2003). They reported low Choice steaks being redder in color than high Choice steaks on d 5, 7, and 10 of display according to trained color panelists. Steaks packaged in HiO_2 MAP were redder than steaks packaged in PVC. They also found differences among muscles and packaging types, with biceps femoris and semitendinosus steaks packaged in HiO_2 MAP having redder lean color scores than steaks packaged in PVC. They also reported that low Choice steaks in both packaging methods had

less discoloration than high Choice and Select steaks on d 5, 7, and 10 of display. Steaks packaged in HiO_2 MAP from low Choice carcasses also had the least amount of discoloration on d 7 and 10 of display. Biceps femoris and semitendinosus steaks packaged in PVC had more discoloration than steaks from those muscles packaged in HiO_2 MAP. Steaks from the semimembranosus were equally discolored in both packaging methods.

Ultra-low oxygen MAP allows for extended shelf life; however, the display color is not appealing to many consumers in such environments. Improved microbial control results from the inclusion of CO_2 in packaging atmospheres. The use of CO has been approved recently by the FDA and USDA for levels up to 0.4% in retail MAP (USFDA, 2004). Products in MAP that include CO have improved beef color stability with extended display time (Luño et al., 1998; Sørheim et al., 1999; Hunt et al., 2004) and improved pork shelf life and color stability (Krause et al. 2003).

Beggan et al. (2004) compared a master pack system containing low oxygen (0.6% residual) and then leaving the film intact, perforating the film, or removing the film for display to the system of packaging subprimals in vacuum and then cutting and overwrapping them with oxygen permeable film. They evaluated beef longissimus, semimebranosus, psoas major, and gluteus medius muscles. They found that longissimus steaks packaged in the low oxygen environment were able to bloom to an acceptable red color after the 3 wk of storage but the other muscles had oxidized to metmyoglobin (most likely due to the 0.6% residual oxygen present in the packages) and were unable to bloom to a red color after storage. They reported that longissimus steaks were able to maintain acceptable color for 3 d of display in comparison to only 2 d for PVC overwrapped longissimus steaks. It is critical to remove all of the oxygen from a low-oxygen MAP environment to prevent and delay formation of metmyoglobin.

The use of CO in the United States was first approved by USFDA (2002) with the master pack method. Meat was stored in a master pack bag that included an atmosphere of 0.4% CO with CO_2 and N_2. Upon placing meat into the retail display case, meat was removed from the master pack environment and exposed only to air (with PVC overwrap). Hunt et al. (2004) compared the use of this CO MAP master pack technology (storage times of 7, 21, or 35 d) with the common PVC overwrap method of displaying meat to determine the effects on color, shelf-life, and microbial growth in different beef muscle steaks and ground beef. They found that all muscles and ground beef evaluated had acceptable initial cherry red color. Color life of

longissimus, superficial semimembranosus, and ground beef exposed to CO decreased as time in storage with CO increased in comparison to meat not exposed to CO. The color life of psoas major and deep semimembranosus muscles stored in CO MAP was equal to or slightly better than meat only exposed to atmospheric oxygen. Furthermore, they reported meat stored in an environment containing CO did not mask spoilage, as there were no packages containing aerobic plate counts of greater than 7 logs.

Jayasingh et al. (2001) found that exposing meat to 5% CO for 24 h and then vacuum packaging the meat allowed for meat to be red in vacuum packaging for at least 5 wk. Brewer et al. (1994) had previously obtained similar results by packaging meat in 100% CO for 1 h prior to vacuum packaging. Jayasingh et al. (2001) was trying to improve safety of the work environment by using a lower percentage of CO and still obtain red steaks in vacuum packaging. They also packaged ground beef in 0.5% CO MAP and reported red color stability for 8 wk. They found microbial loads exceeding 6 log at 5 wk (equal to color shelf life) for steaks packaged in 5% CO MAP for 24 h and then vacuum packaged and at 7 wk for ground beef stored in 0.5% CO MAP. The process of CO MAP treatment in pressurized conditions followed by vacuum packaging would need to be approved by USDA before being used in industry.

Inclusion of CO at 1% in MAP containing 70% O_2 or 24% O_2 or 70% O_2 with no CO (control) added was evaluated for shelf-life, color stability, and sensory analysis of color and odor of beef loin steaks and ground beef (Luño et al., 1998). They found that loin steaks and ground beef packaged in MAP containing 24% O_2 and 1% CO reduced growth of psychrotrophs with 15 d of storage up to 29 d, whereas there were no differences in microbial growth on meat from control packages or those in 70% O_2 and 1% CO. They also reported increased color stability according to a* with inclusion of 1% CO in the packages. Surface metmyoglobin was less for ground beef and loin steaks packaged in MAP containing CO than ground beef and steaks packaged in control MAP from 12 to 29 d of storage. They reported that panelists did not find brown color and found delayed offensive odor formation in samples packaged in CO MAP but did find that brown color and off odor occurred more rapidly with meat packaged in control MAP.

Sørheim et al. (1999) evaluated beef steaks, pork chops, and ground beef packaged in a HiO_2 MAP (70% O_2/ 30%CO_2) or ULO_2CO MAP (0.4% CO/ 60% CO_2/ 40% N_2) and stored at 4 or 8°C for up to 21 d on color stability, microbiology, and the formation of off odor,. They

found the formation of off odor was faster in meat packaged in HiO_2 MAP than in ULO_2CO MAP and for meat stored at 8°C than 4°C. Bright red color was maintained in meat packaged in ULO_2CO MAP, whereas meat packaged in HiO_2 MAP was bright red initially and discolored faster than meat packaged in ULO_2CO MAP. Meat stored at 8°C discolored faster than meat stored at 4°C. Ground beef and pork chops packaged in HiO_2 MAP had higher numbers of *Brochothrix thermosphacta* than similar meat packaged in ULO_2CO MAP but there were no differences in microbial counts due to packaging method in beef steaks. They also reported that all meat stored at 8°C had more bacterial growth than meat stored at 4°C, regardless of packaging method. They concluded that using MAP with CO and excluding O_2 from the package is the best means for extending color stability and shelf-life based on microbiological enumeration for fresh meat packaging.

Modified atmosphere packaging of steaks from five different beef muscles using atmospheric O_2 (20%) and HiO_2 (80%) levels with and without 0.4% CO were evaluated and CO had no effect on color, reducing activity or oxygen consumption (Seyfert et al., 2007). They believe that CO did not have an effect at 0.4% because the oxygen was at such a higher level (both 20 and 80%) and the formation of oxymyoglobin was more preferential than carboxymyoglobin. They reported that HiO_2 improved color stability and reduced variation in color among muscles. They also found that overall the color stability and reducing activity for the muscles was longissimus lumborum > semitendinosus > superficial semimembranosus > psoas major > deep semimembranosus.

The use of MAP with CO in fresh pork has also been reported as beneficial. Martínez et al. (2005) found that fresh pork sausage packaged in 0.3% CO, 30% CO_2, and the balance argon had the highest a* values (redder) and less lipid oxidation after 20 days of storage when compared to other modified atmospheres that included O_2, N_2, and CO_2. Krause et al. (2003) reported greater color stability as determined by a* values and sensory panelists for injected and non-injected fresh pork chops packaged in MAP containing 0.5% CO than pork chops packaged in overwrap, MAP without CO (20% CO_2/ 80% N_2), and vacuum packaging. They did find increased purge in non-injected pork chops packaged in MAP with CO than other packaging methods. Wicklund et al. (2006) reported injection-enhanced pork chops packaged in MAP with CO were redder and had less purge loss than pork chops packaged in HiO_2 MAP with no effect on flavor or consumer acceptability. Wilkinson et al. (2006) compared master-packaged fresh

pork in 100% CO_2 or including 0.4% CO and found improved color with the CO MAP and no effects on microbial counts or lipid oxidation.

COOKED COLOR

The rate of denaturation during cooking is dependent on the oxidation state of myoglobin. Deoxymyoglobin has the most stability to denaturation followed by oxymyoglobin and then metmyoglobin (Machlik, 1965).

Premature Browning

Premature browning results when ground-beef patties cooked to temperatures lower than what is necessary to kill harmful pathogens (and when the meat should still appear red to pink internally) appear well done in internal appearance (Hague et al., 1994). This phenomenon is also found in whole muscle steaks as well (Seyfert et al., 2004b; John et al., 2004).

Visual color of premature-brown ground beef patties at 55°C was similar in visual cooked color to normal patties at 75°C and instrumental color (Warren et al., 1996b). Normal patties were reddest at 55°C and became less red at 65 and 75°C. Premature-brown patties were darkest at 65 and 75°C. These color data are similar to what Marksberry (1990) and Hague et al. (1994) reported. Warren et al. (1996b) found no differences between patties that resulted in premature browning and normal patties for heme and nonheme iron, total pigment, and pH. The patties that prematurely turned brown had higher TBARS values, oxidative-reduction potential, and lower total reducing activity than patties that resulted in normal internal cooked color. They concluded that premature browning is due to patty oxidation and not chemical composition of the patties.

To further show that oxidative state of ground beef patties affects internal cooked color, Warren et al. (1996a) compared patties that were known to develop normal or premature brown internal cooked color. They treated both types of patties with no chemical (control), reduced them with sodium hydrosulfite (reduced), or oxidized them with potassium ferricyanide (oxidized). Internal raw color was purplish red in appearance for the control patties, normal reduced patties, and reduced premature brown patties. Internal raw color was brown for the normal oxidized patties, premature brown oxidized patties, and control patties. Patties were cooked to 55°C and the normal reduced patties were the most red. The normal control patties and premature brown reduced patties were intermediate in color and looked very pink.

Premature brown control and oxidized patties were the least red. There were no differences found among all patties for visual juice color or expressible juice color. They concluded that oxidative state of the patty at time of cooking is critical for the development of internal cooked color and that factors promoting oxidation of myoglobin may result in premature browning during cooking of ground beef patties.

Lavelle et al. (1995) looked at ground beef from steers fed vitamin E supplementation and found that premature browning was not induced or prevented by vitamin E supplementation; however, patties from supplemented steers cooked from the oxidative state would result in premature browning as patties from non-supplemented steers would. They also reported expressible juice color was not affected by vitamin E supplementation.

Van Laack et al. (1996) compared cooked internal color of 17 different commercially prepared ground beef patty formulations cooked to 71°C. They reported differences in red color of beef patties cooked to 71°C due to incomplete denaturation of myoglobin at higher pH values. They reported the need to cook many formulations to > 80°C to obtain a well done appearance. They also stored the cooked patties in vacuum packaging in the freezer (-27°C) for 1 yr and found 16 of the 17 treatments that had been cooked to 71°C were more pink/red after storage than prior to storage. The changes in cooked color after storage could not be explained by changes in reducing activity or metmyoglobin content, and they could not explain the changes observed.

Hunt et al. (1999) cooked ground beef patties with deoxymyoglobin, oxymyoglobin, or metmyoglobin as the primary internal pigment to 55, 65, or 75°C from a thawed or frozen state. They showed that only patties in the deoxymyoglobin form did not premature brown and had more thermal stability than patties containing mostly metmyoglobin or oxymyoglobin. They also reported that, as pH increased, the thermostability of metmyoglobin and oxymyoglobin increased but never to the point of deoxymyoglobin. Hunt et al. (1999) confirmed the thermostability research of Machlik (1965) and also determined that pigment form and pH of the meat affect internal cooked color.

Retail purchasing of ground beef for patty making had an average of 47% incidence of premature browning (Killinger et al., 2000). They found that location of ground beef within package, storage conditions, and time of purchase all affect formation of premature browning when patties were cooked to 55°C. Patties that were made from the outer portion of the

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packages (most oxymyoglobin) from packages purchased approximately 2 hr after the stores'

morning grinding had 62.5% of the patties turn premature brown when cooked to 55°C. Patties

formed from the inner part of the package (more deoxymyoglobin) and purchased in the

afternoon and stored overnight became prematurely brown when cooked to 55°C in 25% of the

samples. This study provided further evidence that cooked color cannot be used as a safe and

effective way to determine doneness in ground beef patties and that internal temperature needs to

be monitored (including consumers) to correctly determine doneness of ground beef.

Suman et al. (2004) compared longissimus lumborum and psoas major muscles to form

ground beef patties and found that after 0, 48, or 96 h of storage patties made from the

longissimus lumborum had more stable internal cooked redness than patties made from the psoas

major, which had decreased redness with increased storage of patties. They suggested that

separating muscles and making ground beef patties from muscles that maintain cooked red color

may be useful in preventing premature browning.

Packaging Affects Premature Browning

Different types of packaging also can change internal cooked color. Seyfert et al.

(2004b,c) determined that beef packaged in HiO_2 MAP was prematurely brown at a medium

degree of doneness. Seyfert et al. (2004b) evaluated biceps femoris, semimembranosus, vastus

lateralis, and rectus femoris muscles injection-enhanced to 6% and steaks packaged in HiO_2

MAP or ULO_2 MAP and then cooked to 71.1°C. The internal raw color of steaks packaged in

HiO_2 MAP was oxymyoglobin and the interior of steaks packaged in ULO_2 MAP was

deoxymyoglobin. They found that steaks packaged in HiO_2 MAP had a well-done appearance at

71.1°C when a pinkish internal color was the typical internal cooked color at this medium degree

of doneness. In contrast to steaks packaged in HiO_2 MAP, steaks packaged in ULO_2 MAP and

cooked to 71.1°C had a pinkish-red interior as expected of meat cooked to a medium degree of

doneness. Both visual and instrumental color data supported these findings. Differences in

cooked color among muscles were minor in comparison to differences in cooked color due to

packaging treatment. They also reported that steaks packaged in HiO_2 MAP had 93.8%

denatured myoglobin after cooking, whereas steaks packaged in ULO_2 MAP had 57.8% thermal

denaturation of myoglobin.

The concern for premature browning as a result of packaging type may be even greater for ground beef. Seyfert et al. (2004c) looked at ground beef patties packaged in HiO_2 MAP or vacuum packaging at different days of display (3, 5, 8, 10, 12, and 14) and reported that nearly 100% of patties made from ground beef packaged in HiO_2 MAP were prematurely brown at 71.1°C. Patties made from ground beef packaged in HiO_2 MAP using the top portion or bottom portion of meat in the package were susceptible to premature browning. Patties made from ground beef packaged in vacuum packaging did not prematurely turn brown and had a pinkish center at 71.1°C that actually became more intense in red color after initial color measurements because the myoglobin was not completely denatured in these samples and bound to atmospheric oxygen to intensify the red color. Cooked color is not an indicator of doneness in ground beef or meat packaged in HiO_2 MAP and could pose safety risks if consumers do not properly use a thermometer to monitor and determine endpoint temperature.

Ground beef patties made from chubs of ground beef packaged in 80% O_2, 0.4% CO, or vacuum packaged stored 7, 14, or 21 d and cooked to 49, 57, 66, 71, or 79°C internal temperature were evaluated for color, TBARS, and myoglobin denaturation (John et al., 2004). They found that patties made from beef packaged in 80% oxygen had higher TBARS values, more myoglobin denaturation, and higher internal cooked color that was less red than patties made from beef packaged in 0.4% CO or vacuum packaged. Patties made from beef stored in 0.4% CO had persistent pinking when cooked to 79°C and the authors attributed this to the likely formation of heat-denatured CO-hemochrome and not undenatured CO myoglobin.

John et al. (2005) compared top sirloin steaks packaged in 80% O_2, 0.4% CO, or vacuum after 7, 14, or 21 days of storage and at internal temperatures of 49, 57, 66, 71, or 79°C. They found that steaks packaged in 80% oxygen had the most oxidation, according to TBARS values, and greatest myoglobin denaturation at all cooking endpoint temperatures and storage times. Steaks packaged in 80% oxygen also became prematurely brown at 57°C although internal cooked color did not turn completely brown until steaks were cooked to 66°C. Steaks packaged in 0.4% CO or vacuum packaging maintained some pinkish red internal cooked color through 79°C.

INJECTION ENHANCEMENT OF MEAT

The use of 'injection enhancement' has been shown to improve beef tenderness and juiciness and typically is used in conjunction with case-ready, HiO_2 MAP. Beef round muscles injected 10% with a solution containing salt, phosphate, and natural flavoring solution had less oxidation but more non-typical beef flavors than muscles injected with a 6% enhancement solution (Seyfert et al., 2005). They found that beef quadriceps muscles packaged in HiO_2 MAP were less tender and had more off-flavors than those packaged in ULO_2 MAP. Steaks packaged in ULO_2 MAP had increased beef flavor and had less off flavors. Common descriptors of off flavors they found in muscles packaged in HiO_2 MAP were oxidative and rancid. Muscles packaged in both ULO_2 and HiO_2 MAP were also described as salty, sour, and bitter. They reported higher TBARS values for injection-enhanced steaks packaged in HiO_2 MAP than steaks packaged in ULO_2 MAP. According to trained sensory panelists, steaks packaged in ULO_2 MAP had increased myofibrillar and overall tenderness as well as decreased perception of connective tissue than steaks packaged in HiO_2 MAP. They suggest some of the differences in tenderness may be due to the differences in aging time, as steaks packaged in HiO_2 MAP were frozen for sensory analysis at 14 d postmortem and steaks packaged in ULO_2 MAP were frozen at 22 d postmortem.

In another study, beef rib steaks were injected with different combinations of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate and packaged in HiO_2 MAP (Knock et al., 2006b). They found that steaks from muscles injected with potassium lactate with or without sodium acetate had increased color stability but were darker than control steaks. They also reported less surface glossiness or shine when the enhancement solution contained sodium lactate or acetate than control steaks. They showed increased salt content was detrimental to visual color of steaks but did reduce surface shine. They also reported increased TBARS values with increased time steaks were displayed in HiO_2 MAP, with a greater increase from d 9 to d 14 of display. In a similar study looking at injected beef strip loin steaks, Knock et al. (2006a) found that adding potassium lactate to injection-enhanced beef packaged in HiO_2 MAP limited rancid flavor development while increasing brown-roasted and beef flavors. They also found that increasing the salt content in the injection-enhancement solution increased salty and rancid flavors and oxidized, stale and rancid flavors increased as time in HiO_2 MAP increased. They also reported that steaks enhanced with sodium acetate had lower shear force

than control steaks or steaks enhanced with potassium lactate but the mechanism for this tenderness difference is unknown.

Injection of beef Select and Choice strip loins with solutions containing sodium lactate, sodium chloride, and sodium tripolyphosphate at varying levels from 107.5 to 115% of the raw weight were evaluated for WBSF and sensory attributes (Vote et al., 2000). They reported increased tenderness and juiciness for injected samples compared to control, non-injected steaks. They also found a trend for increased cooked beef flavor, but when sodium tripolyphosphate was injected alone, soapy and sour off-flavors were detected. They also cooked steaks to either 66 or 77°C and showed that the improvements in tenderness and juiciness compared to control steaks were even greater at 77°C. Thus, injection enhancement may be beneficial when consumers overcook steaks, and it helps processors deliver more consistent products to their consumers.

The use of antioxidants in enhancement solutions has been tried. Adding an oregano-oil brine enhancement to beef steaks packaged in HiO_2 MAP decreased lipid oxidation but significantly increased off flavor and overall acceptability when compared to control samples (Scramlin et al., 2006). Enhancing beef with salt, BHA/BHT and phosphates had similar flavor to control samples but did increase juiciness and decrease the amount of connective tissue (Rowe, et al., 2006).

Lawrence et al. (2003a) evaluated injection marination of beef longissimus muscles with 0.1, 0.2, or 0.3 M solutions of calcium ascorbate, calcium chloride, or calcium lactate for effects on display color, shelf-life, shear force, and sensory attributes. They reported that calcium lactate increased beef flavor and had no effect on off flavor of injection-enhanced beef strip loins compared to control samples and recommend using it as a 0.1 M solution and not using calcium ascorbate or calcium chloride. Lawrence et al. (2003b) injected beef longissimus and semitendinosus muscles with calcium lactate followed by a phosphate and salt injection solution. They found no differences in tenderness or sensory traits for the semitendinosus muscle but found increased tenderness for injected longissimus muscles compared to control muscles. They reported longissimus steaks from muscles injected with phosphate and salt in addition to calcium lactate had less beef flavor and complaints of samples being too salty than steaks from control muscles or muscles only injected with calcium lactate. In a further study, Lawrence et al. (2004) evaluated injection of beef longissimus muscles with a phosphate and salt solution or a calcium lactate plus beef broth or carrageenan with rosemary extract solution. They reported

improvements in display color stability when rosemary extract was included in the solution with calcium lactate than steaks injected with phosphate and salt with rosemary. They found no differences in WBSF among all treatments. According to trained sensory panelists, steaks enhanced with calcium lactate with rosemary were less tender than steaks enhanced with phosphate and salt with rosemary, but off flavors of metallic and salty were increased with the phosphate and salt with rosemary treatment compared to the calcium lactate with rosemary treatment.

Enhancement of cow longissimus and semitendinosus muscles with a commercial salt, sodium and potassium phosphates, and lactate solution after 7 d of aging, and then an additional 7 d of aging after enhancement, was evaluated for sensory traits (Hoffman, 2006). They reported longissimus and semitendinosus steaks were more tender and juicier than non-enhanced steaks. They also found that enhanced steaks were saltier and had less overall beef flavor than longissimus and semitendinosus steaks that were non-enhanced.

Papadopoulos et al. (1991b) injected beef round roasts with 0, 1, 2, 3, or 4 % sodium lactate and 0.5% sodium chloride and had trained sensory panelists evaluate samples from d 0 to 84 of frozen storage on cooked product. They reported increased cook yields with increasing percentages of sodium lactate along with a slowed decreased of "on-notes," including fresh beef flavor, and decreased incidence of warmed-over flavor. With the exception of some mild throat irritations from samples containing 4% sodium lactate and stored less than 56 d, the authors did not report adverse effects on sensory properties due to the inclusion of sodium lactate. In a similar study, Papadopoulos et al., (1991a) evaluated beef trop rounds injected with salt, sodium tripolyphosphate, and 0 or 3% sodium lactate. They reported 'positive flavor notes,' such as cooked beefy, brothy, and bloody-serumy associated with beef top rounds enhanced with sodium lactate, but these positive flavor notes decreased with storage time. A consumer panel found that lactate enhanced beef to have better overall flavor, beef flavor, and texture, but they also reported increased saltiness and sourness with lactate enhanced beef compared to control samples. They also found decreased warmed-over flavor with lactate enhanced beef. In addition, samples from rounds injected with lactate had lower aerobic plate counts after 42 and 84 d of storage than control samples.

Maca et al. (1997) injected beef top round roasts with solutions containing sodium chloride, sodium tripolyphosphate and sodium lactate (3 or 4%) with or without sodium

propionate (0.1 or 0.2%) and stored them cooked from 0 to 84 d. They reported decreased aerobic plate counts in injected roasts (any combination of ingredients) compared to control roasts. They found increased beefy flavors in roasts injected with both sodium lactate and propionate on d 0 and 14 of storage in comparison to samples from non-injected roasts. They also reported increased sour, salty, and bitter flavors with storage. They recommend not exceeding 3% sodium lactate in combination with 0.1% sodium propionate when injecting beef round roasts.

Wicklund et al. (2005) compared injection-enhancement (phosphate, salt, natural flavoring) of beef strip loins before and after aging for 7, 14, 21, or 28 d in vacuum packaging. They found that enhanced steaks were saltier than non-enhanced steaks, but aging had no effect on salty flavor. They reported that enhanced steaks were juicier than non-enhanced steaks, with steaks that were enhanced after aging having no change in juiciness compared to those steaks that were enhanced prior to aging; enhancement prior to aging decreased juiciness as storage time increased. They also showed that enhanced steaks were more tender than non-enhanced steaks according to trained sensory panelists, with enhanced steaks achieving maximal tenderness by 14 d of aging and non-enhanced samples reaching maximal tenderness after 21 d of aging. They reported that steaks enhanced before aging had lower WBSF values than non-enhanced steaks by d 7 of aging, but there was no decrease in WBSF of enhanced samples after aging, although all enhanced steaks were more tender than non-enhanced steaks. Thus, it appears that enhancement treatment increases tenderness, regardless if the enhancement process takes place prior to or after aging the muscles. They also found that enhanced steaks were darker in color and less red and yellow than non-enhanced steaks.

Molina et al. (2005) compared marinating, needle-pumping, and vacuum-tumbling with a salt phosphate solution to control samples from eight muscles from the chuck and saw decreased or no change in beef flavor intensity but did find increased juiciness and tenderness in several of the muscles. Seven of the 8 muscles had increased off-flavors associated with marinating compared to needle-pumping or vacuum-tumbling. Overall, they could not find a consistent effect in the eight muscles evaluated in terms of sensory attributes. They reported lower WBSF values for needle-pumped complexus and triceps brachii muscles than control samples, and all three treatment processes decreased WBSF in the subscapularis compared to the control.

Robbins et al. (2003b) compared beef steaks that were enhanced or non-enhanced from vitamin E supplemented cattle or cattle that received no supplementation. They reported that enhanced steaks were more tender, juicy, and salty than controls by trained sensory panelists. In contrast to other studies, Robbins et al. (2003b) found no difference in beefy flavor or aftertaste. They did not find any effect on supplementing vitamin E to cattle for sensory properties but did find that supplementation of vitamin E decreased brown color intensity of fresh beef steaks and improved color stability of non-enhanced steaks compared to steaks from cattle not supplemented with vitamin E. They reported that enhanced steaks were darker in color than non-enhanced steaks.

When comparing enhanced and non-enhanced beef roasts from cattle receiving vitamin E supplementation or no supplementation served hot or held in heat to stay warm for 1 and 2 h, there was increased beef flavor, juiciness, saltiness, and tenderness in enhanced samples (Robbins, et al., 2003c). They reported that enhanced roasts had more beef/brothy aroma than non-enhanced roasts. They found that enhanced samples were juicier than non-enhanced samples, but samples from non-supplemented cattle that were enhanced were juicier than supplemented cattle that were enhanced.

Beef steaks and roasts from enhanced or non-enhanced muscles from cattle fed vitamin E supplementation or no vitamin E supplementation were evaluated for consumer acceptability (Robbins et al., 2003a). They reported no positive effects on sensory scores of steaks or roasts from cattle fed vitamin E, but consumers found that steaks from non-supplemented cattle to be more salty and have increased beef flavor than steaks from cattle fed vitamin E. Consumer acceptability was found to be greater in enhanced beef compared to control, non-enhanced beef. Consumers found that enhanced steaks and roasts were more tender and juicy, and have increased beef flavor, saltiness, and overall acceptability compared to control steaks. Additionally, they reported that purchasing decisions were based on color, price, visible fat, and cut, whereas eating satisfaction was based on tenderness, flavor, and juiciness. Consumers also indicated a concern about added ingredients.

Stetzer et al. (2007) compared beef loin steaks injection-enhanced with phosphate, salt, and natural flavorings and packaged in HiO_2 MAP or ULO_2CO MAP and reported that consumer sensory panelists did not find differences in beef flavor, off flavor, or overall acceptability due to MAP. They also reported no differences in purge loss or cook loss when steaks in both

packaging methods were stored for 14 d, but steaks in CO MAP had more cooking loss when stored for 28 d compared to steaks in both packaging types at 14 d of storage. Trained color panelists found that steaks packaged in HiO_2 MAP were redder than steaks packaged in CO MAP. Furthermore, their trained color panelists observed no differences in CO MAP steak internal cooked color when stored for 14 d in HiO_2 MAP or 28 d in CO MAP, but did find that steaks in CO MAP stored for 14 d were browner.

SUMMARY

Generally, enhanced beef is found to be juicier and more tender; however, there appears to be inconsistencies in the effects of enhancement on beef flavor intensity and off flavors, with some studies showing off-flavors in enhanced products. Packaging of enhanced beef in HiO_2 MAP tends to increase oxidative and/or rancid off flavors. With the goal of consistently providing consumers with tender, juicy, and flavorful beef, current enhancement strategies may increase tenderness and juiciness but, at the same time, may not adequately represent good beef flavor and, in some cases, typical beef texture. An additional problem associated with enhanced beef is the health concern of providing large amounts of sodium (one serving providing approximately 12% of recommended daily sodium allowance) to the diet.

It is important for the beef industry to provide and promote good tasting beef that results in a pleasurable eating experience that will, in turn, ensure repeat purchases and consumption of beef. In addition, it is important that the right muscles are enhanced in the right way, and that value is increased but not costs.

LITERATURE CITED

Baran, W. L., A. A. Kraft, and H. W. Walker. 1970. Effects of carbon dioxide and vacuum packaging on color and bacterial count of meat. J. Milk Food Technol. 33:77-82.

Beggan, M., P. Allen, and F. Butler. 2004. Shelf-life of retail beef muscles following storage in a low oxygen environment. J. Mus. Foods. 15:269-285.

Behrends, J. M., W. B. Mikel, C. L. Armstrong, and M. C. Newman. 2003. Color stability of semitendinosus, semimembranosus, and biceps femoris steaks packaged in a high-oxygen modified atmosphere. J. Anim. Sci. 81:2230-2238.

Brewer, M. S., S. Wu, R. A. Field, and B. Ray. 1994. Carbon monoxide effects on color and microbial counts of vacuum packaged beef steaks in refrigerated storage. J. Food Quality. 17:231-236.

Campo, M. M., G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson. 2006. Flavour perception of oxidation in beef. Meat Sci. 72:303-311.

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

Clausen, I. 2004. Sensory evaluation of beef loin steaks stored in different atmospheres. Proc. 50th Int. Congr. Meat Sci. Technol. Helsinki, Finland.

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

Dikeman, M. E. 1987. Fat reduction in animals and the effects on palatability and consumer acceptance of meat products. Proc. 40th Recip. Meat Conf.:93.

Eilert., S. J. 2005. Review: New packaging technologies for the 21st century. Meat Sci. 71:122-127.

George, M. H., J. D. Tatum, K. E. Belk, and G. C. Smith. 1999. An audit of retail beef loin steak tenderness conducted in eight U.S. cities. J. Anim. Sci. 77:1735-1741.

Grobbel, J. P., M. E. Dikeman, E. J. Yancey, J. S. Smith, D. H. Kropf, and G. A. Milliken. 2006. Effects of ascorbic acid, rosemary, and Origanox™ in preventing bone marrow discoloration in beef lumbar vertebrae in aerobic and anaerobic packaging systems. Meat Sci. 72(1):47-56.

Hague, M. A., K. E. Warren, M. C. Hunt, D. H. Kropf, C. L. Kastner, S. L. Stroda, and D. E. Johnson. 1994. Endpoint temperature, internal cooked color, and expressible juice color relationships in ground beef patties. J. Food Sci. 58:465-470.

Hoffman, L. C. 2006. Sensory and physical characteristics of enhanced vs. non-enhanced meat from mature cows. Meat Sci. 72:195-202.

Hunt, M. C., O. Sørheim, and E. Slinde. 1999. Color and heat denaturation of myoglobin forms in ground beef. J. Food Sci. 64(5):847-851.

Hunt, M. C., R. A. Mancini, K. A. Hachmeister, D. H. Kropf, M. Merriman, G. DelDuca, and G. Milliken. 2004. Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorganisms of beef steaks and ground beef. J. Food Sci. 69(1):C45-C52.

Jackson, T. C., G. R. Acuff, C. Vanderzant, T. R. Sharp, and J. W. Savell. 1992. Identification and evaluation of volatile compounds of vacuum and modified atmosphere packaged beef strip loins. Meat Sci. 31:175-190.

Jayasingh, P., D. P. Cornforth, C. E. Carpenter, and D. Whittier. 2001. Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. Meat Sci. 59:317-324.

Jeyamkóndan, S., D. S. Jayas, and R. A. Holley. 2000. Review of centralized packaging systems for distribution of retail-ready meat. J. Food Prot. 63:796-804.

John, L., D. Cornforth, C. E. Carpenter, O. Sørheim, B. C. Pettee, and D. R. Whittier. 2004. Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. J. Food Sci. 69(8):C608-C614.

John, L., D. Cornforth, C. E. Carpenter, O. Sorheim, B. C. Pettee, and D. R. Whittier. 2005. Color and thiobarbituric acid values of cooked top sirloin steaks packaged in modified atmospheres of 80% oxygen, or 0.4% carbon monoxide, or vacuum. Meat Sci. 69:441-449.

Kannan, G., C. B. Chawan, B. Kouakou, and S. Gelaye. 2002. Influence of packaging method and storage time on shear values and mechanical strength of intramuscular connective tissue of chevon. J. Anim. Sci. 80:2383-2389.

Killinger, K. M., M. C. Hunt, R. E. Campbell, and D. H. Kropf. 2000. Factors affecting premature browning during cooking of store-purchased ground beef. J. Food Sci. 65(4):585-587.

Knock, R. C., M. Seyfert, M. C. Hunt, M. E. Dikeman, R. A. Mancini, J. A. Unruh, J. J. Higgins, and R. A. Monderen. 2006a. Effects of potassium lactate, sodium chloride, and sodium acetate on surface shininess/gloss and sensory properties of injection-enhanced beef strip-loin steaks. Meat Sci. 74:319-326.

Knock, R. C., M. Seyfert, M. C. Hunt, M. E. Dikeman, R. A. Mancini, J. A. Unruh, J. J. Higgins, and R. A. Monderen. 2006b. Effects of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate on colour, colour stability, and oxidative properties of injection-enhanced beef rib steaks. Meat Sci. 74:312-318.

Koohmaraie, M. 1994. Muscle proteinases and meat aging. Meat Sci. 36:93-104.

Koohmaraie, M. and G. H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci. 74(1):34-43.

Krause, T. R., J. G. Sebranek, R. E. Rust, and M. S. Honeyman. 2003. Use of carbon monoxide packaging for improving the shelf life of pork. J. Food Sci. 68(8):2596-2603.

Kropf, D. H. 2000. Meat, modified atmosphere packaging. Page 1561-1567 in Encyclopedia of Food Science & Technology. John Wiley, Indianapolis.

Kropf, D. H. 2004. Packaging. Page 943-969 in Encyclopedia of Meat Sciences. W. K. Jensen, C. Devine and M. E. Dikeman eds. Elsevier Ltd., Oxford.

Lavelle, C. L., M. C. Hunt, and D. H. Kropf. 1995. Display life and internal cooked color of ground beef from vitamin E-supplemented steers. J. Food Sci. 60(6):1175-1178, 1196.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003a. Effects of calcium salts on beef longissimus quality. Meat Sci. 64(2):299-308.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2004. Effects of enhancing beef *longissimus* with phosphate plus salt, or calcium lactate plus non-phosphate water binders plus rosemary extract. Meat Sci. 67:129-137.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003b. Staged injection marination with calcium lactate, phosphate and salt may improve beef water-binding ability and palatability traits. Meat Sci. 65:967-972.

Lee, M., J. Sebranek, and F. C. Parrish. 1996. Accelerated postmortem aging of beef utilizing electron-beam irradiation and modified atmosphere packaging. J. Food Sci. 61(1):133-136, 141.

Luño, M., J. A. Beltrán, and P. Roncalés. 1998. Shelf-life extension and colour stabilization of beef packaged in a low O2 atmosphere containing CO: loin steaks and ground meat. Meat Sci. 48(1/2):75-84.

Maca., J. V., R. K. Miller, J. D. Maca, and G. R. Acuff. 1997. Microbiological, sensory and chemical characteristics of vacuum-packaged cooked beef top rounds treated with sodium lactate and sodium propionate. J. Food Sci. 62(3):586-590, 596.

Machlik, S. M. 1965. The effect of heat on bovine myoglobin derivatives in model systems and in beef semitendinosus muscle. Ph.D. Dissertation, Purdue University, West Lafayette, IN.

Madsen, N. T., and I. Clausen. 2006. Sensory evaluation of beef steaks stored in MA combinations of CO, CO2, N2, and O2. Page 405-406 in Proc 52nd Int. Congr. Meat Sci. Technol., Dublin, Ireland.

Mancini, R. A., and M. C. Hunt. 2005. Review: Current research in meat color. Meat Sci. 71:100-121.

Marksberry, C. L. 1990. The effect of fat level, pH, carcass maturity, and compaction on the cooked internal color of ground beef patties at five endpoint temperatures. M.S. Thesis. Kansas State University, Manhattan.

Martínez, L., D. Djenane, I. Cilla, J. A. Beltrán, and P. Roncalés. 2005. Effect of different concentrations of carbon dioxide and low concentration of carbon monoxide on the shelf-life of fresh pork sausages packaged in modified atmosphere. Meat Sci. 71:563-570.

McKenna, D. R., P. D. Mies, B. E. Baird, K. D. Pfeiffer, J. W. Ellebracht, and J. W. Savell. 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat Sci. 70:665-682.

Miller, M. F., K. L. Huffman, S. Y. Gilbert, L. L. Hammon, and C. B. Ramsey. 1995. Retail consumer acceptance of beef tenderized with calcium chloride. J. Anim. Sci. 73:2308.

Miller, M. F., M. A. Carr, C. B. Ramsey, K. L. Crockett, and L. C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062-3068.

Mintert, J., J. L. Lusk, T. C. Schroeder, J. A. Fox, and M. Koohmaraie. 2000. Valuing beef tenderness. Kansas State University, Manhattan, KS.

Mize, J., and J. Kelly. 2004. America's dynamic meat case. Cryovac Retail Wrap-up, December. Cryovac Sealed Air Corp., Duncan, SC

Molina, M. E., D. D. Johnson, R. L. West, and B. L. Gwartney. 2005. Enhancing palatability traits in beef chuck muscles. Meat Sci. 71:52-61.

Papadopoulos, L. S., R. K. Miller, G. R. Acuff, L. M. Lucia, C. Vanderzant, and H. R. Cross. 1991a. Consumer and trained sensory comparisons of cooked beef top rounds treated with sodium lactate. J. Food Sci. 56(3):621-626, 635.

Papadopoulos, L. S., R. K. Miller, L. J. Ringer and H. R. Cross. 1991b. Sodium lactate effect on sensory characteristics, cooked meat color, and chemical composition. J. Food Sci. 56(3):621-626, 635.

Robbins, K., J. Jensen, K. J. Ryan, C. Homco-Ryan, F. K. McKeith, and M. S. Brewer. 2003a. Consumer attitudes towards beef and acceptability of enhanced beef. Meat Sci. 65:721-729.

Robbins, K., J. Jensen, K. J. Ryan, C. Homco-Ryan, F. K. McKeith, and M. S. Brewer. 2003b. Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks. Meat Sci. 64:279-285.

Robbins, K., J. Jensen, K. J. Ryan, C. Homco-Ryan, F. K. McKeith, and M. S. Brewer. 2003c. Effects of dietary vitamin E supplementation on textural and aroma attributes of enhanced beef clod roasts in a cook/hot-hold situation. Meat Sci. 64:317-322.

Rowe, L. J., K. R. Maddock, S. M. Lonergan, and E. Huff-Lonergan. 2004a. Influence of early postmortem protein oxidation in beef quality. J. Anim. Sci. 82:785-793.

Rowe, L. J., K. R. Maddock, S. M. Lonergan, and E. Huff-Lonergan. 2004b. Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. J. Anim. Sci. 82:3254-3266.

Rowe., C. W., F. W. Pohlman, A. H. Brown, Jr., R. T. Bauublits, and Z. B. Johnson. 2006. Effects of salt, BHA/BHT and differing phosphate types on quality and sensory characteristics of beef longissimus muscles. Page 5 in Proc. 59th Recip. Meat Conf. Urbana, IL.

Scramlin, S. M., M. C. Newman, R. B. Cox, H. A. Sepe, A. L. Alderton, J. O'Leary, and W. B. Mikel. 2006. Effects of oregano oil brine enhancement, on quality attributes of beef longissimus dorsi and semimembranosus muscles from various age animals. Page 42-43 in Proc. 59th Recip. Meat Conf. Urbana, IL.

Sekar, A., K. Dushyanthan, K. T. Radhakrishnan, R. Narendra Babu. 2006. Effect of modified
atmosphere packaging on structural and physical changes in buffalo meat. Meat Sci.
72:211-215.

Seyfert, M. M. C. Hunt, R. A. Mancini, K. A. Hachmeister, D. H. Kropf, and J. A. Unruh. 2004a.
Accelerated chilling and modified atmosphere packaging affect colour and colour
stability of injection-enhanced beef round muscles. Meat Sci. 68:209-219.

Seyfert, M., M. C. Hunt, R. A. Mancini, D. H. Kropf, and S. L. Stroda. 2004b. Internal
premature browning in cooked steaks from enhanced beef round muscles packaged in
high-oxygen and ultra-low oxygen modified atmospheres. J. Food Sci. 69(2):FCT142-
FCT146.

Seyfert, M., M. C. Hunt, R. A. Mancini, K. A. Hachmeister, D. H. Kropf, J. A. Unruh, and T. M.
Loughin. 2005. Beef quadriceps hot boning and modified atmosphere packaging
influence properties of injection-enhanced beef round muscles. J. Anim. Sci. 83:686-693.

Seyfert, M., R. A. Mancini, and M. C. Hunt. 2004c. Internal premature browning in cooked
ground beef patties from high-oxygen modified-atmosphere packaging. J. Food Sci.
69(9):C721-C725.

Seyfert, M., R. A. Mancini, M. C. Hunt, J. Tang, and C. Faustman. 2007. Influence of carbon
monoxide in package atmospheres containing oxygen on colour, reducing activity, and
oxygen consumption of five bovine muscles. Meat Sci. 75:432-442.

Seyfert, M., R. A. Mancini, M. C. Hunt, J. Tang, C. Faustman, and M. Garcia. 2006. Color
stability, reducing activity, and cytochrome *c* oxidase activity of five bovine muscles. J.
Ag. Food Chem. 54:8919-8925.

Smulders, F. J. M., J. Hiesberger, P. Hofbauer, B. Dögl, and E. Dransfield. 2006. Modified-
atmosphere storage under subatmospheric pressure and beef quality: II. Color, drip,
cooking loss, sarcomere length, and tenderness. J. Anim. Sci. 84:2456-2462.

Sørheim, O., H. Nissen, and T. Nesbakken. 1999. The storage life of beef and pork packaged in
an atmosphere with low carbon monoxide and high carbon dioxide. Meat Sci. 52:157-
164.

Sørheim, O., M. Wahlgren, B. N. Nilsen, and P. Lea. 2004. Effects of high oxygen packaging on
tenderness and quality characteristics of beef longissimus muscles. Proc. 50th Int. Congr.
Meat Sci. Technol., Helsinki, Finland.

Stetzer, A. J., R. A. Wicklund, D. D. Paulson, E. M. Tucker, B. J. MacFarlane, and M. S. Brewer. 2007. Effect of carbon monoxide and high oxygen modified atmosphere packaging (MAP) on quality characteristics of beef strip steaks. J. Mus. Foods. 18:56-66.

Suman, S. P., C. Faustman, S. Lee, J. Tang, H. A. Sepe, P. Vasudevan, T. Annamalai, M. Manojkumar, P. Marek, M. DeCesare, K. S. Venkitanarayanan. 2004. Effect of muscle source on premature browning in ground beef. Meat Sci. 68:457-461.

Tørngren, M. A. 2003. Effect of packing method on colour and eating quality of beef loin steaks. Pages 495-496 in Proc. 49th Int. Congr. Meat Sci. Technol., Campinas, Brazil.

U. S. Food and Drug Administration (USFDA). 2002. GRAS notice number GRN 000083. Washington D.C.

U. S. Food and Drug Administration (USFDA). 2004. GRAS notice number GRN 000143. Washington, D.C.:

Van Laack, R. L. J. M., B. W. Berry, and M. B. Solomon. 1996. Variations in internal color of cooked beef patties. J. Food Sci. 61(2):410-414.

Vote, D. J., W. J. Platter, J. D. Tatum, G. R. Schmidt, K. E. Belk, G. C. Smith, and N. C. Speer. 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. J. Anim. Sci. 78:952-957.

Warren, K. E., M. C. Hunt, and D. H. Kropf. 1996a. Myoglobin oxidative state affects internal cooked color development in ground beef patties. J. Food Sci. 61(3):513-515, 519.

Warren, K. E., M. C. Hunt, D. H. Kropf, M. A. Hague, C. L. Waldner, S. L. Stroda, and C. L. Kastner. 1996b. Chemical properties of ground beef patties exhibiting normal and premature brown internal cooked color. J. Mus. Foods. 7:303-314.

Wicklund, S. E., C.Homco-Ryan, K. J. Ryan, F. K. McKeith, B. J. McFarlane, and M. S. Brewer. 2005. Aging and enhancement effects on quality characteristics of beef strip steaks. J. Food Sci. 70(3):S242-S248.

Wicklund, R. A., D. D. Paulson, E. M. Tucker, A. J. Stetzer, F. DeSantos, M. Rojas, B. J. MacFarlane, and M. S. Brewer. 2006. Effect of carbon monoxide and high oxygen modified atmosphere packaging and phosphate enhanced, case-ready pork chops. Meat Sci. 74:704-709.

Wilkinson, B. H. P., J. A. M. Janz, P. C. H. Morel, R. W. Purchas, and W. H. Hendriks. 2006. The effect of modified atmosphere packaging with carbon monoxide on the storage quality of master-packaged fresh pork. Meat Sci. 73:605-610.

CHAPTER 2 - Effects of Packaging Atmospheres on Beef Instrumental Tenderness, Fresh Color Stability, and Internal Cooked Color

ABSTRACT

Fresh meat color is a major factor influencing consumers' purchases of meat products. Tenderness is the primary trait determining consumers' overall eating satisfaction. The objectives of this research were to determine the effects of packaging atmosphere on fresh beef color stability, cooked color, and tenderness. Longissimus lumborum muscles (n=14 pairs) from USDA Select, A-maturity carcasses were assigned to either 14 d tenderness measurement or to display and then 18 or 28 d tenderness measurement. Loins were fabricated on d 7 postmortem into 2.54 cm-thick steaks. Steaks 8-10 posterior to the first 7 steaks were cut in half, assigned to a packaging treatment, and used for internal cooked color. One full steak was used for initial tenderness. Packaging treatments were: vacuum packaging (VP); 80% O_2/20% CO_2 (HiO$_2$); 0.4% CO/35% CO_2/64.6% N_2 (ULO$_2$CO); 0.4% CO/99.6% CO_2 (ULO$_2$COCO$_2$); 0.4% CO/99.6% N_2 (ULO$_2$CON$_2$); or 0.4% CO/99.6% Ar (ULO$_2$COAr). Steaks packaged in HiO$_2$ modified atmosphere packaging (MAP) were in dark storage (2°C) for 4 d and all other steaks for 14 d. Steaks were displayed under fluorescent lighting (2153 lux, 3000 K) for 7 d with instrumental color measured on d 0 and 7 of display. Trained color panelists (n=10) assigned color scores. Steaks for Warner-Bratzler shear force (WBSF) and cooked color were cooked to 70°C. Steaks packaged in the four ULO$_2$ MAP blends with CO had no change ($P > 0.05$) or increased ($P < 0.05$) a* values for fresh color. Steaks packaged in VP or the four ULO$_2$ MAP blends with CO had little or no surface discoloration. Steaks packaged in HiO$_2$ MAP discolored faster ($P < 0.05$) and 56% more ($P < 0.05$) than those in any other packaging treatment. There were no differences ($P > 0.05$) in WBSF on d 14 postmortem. Steaks packaged in HiO$_2$ MAP were less tender ($P < 0.05$) than other treatments at the end of display, but had 10 d less aging due to a shorter dark storage period. Steaks packaged in HiO$_2$ had the lowest ($P < 0.05$) a* values for internal cooked color of all packaging treatments. Steaks packaged in ULO$_2$COCO$_2$ and VP had intermediate a* values while those packaged in ULO$_2$COAr, ULO$_2$CO, and ULO$_2$CON$_2$ had

the highest ($P < 0.05$) a* values. Ultra-low oxygen packaging treatments had longer fresh color stability than steaks packaged in HiO$_2$ MAP and equal or better tenderness. Packaging atmospheres altered internal cooked color, with steaks packaged in HiO$_2$ MAP exhibiting premature browning.

Key Words: beef, cooked color, display color, modified atmosphere packaging, tenderness

INTRODUCTION

Several meat quality traits are involved in consumers' overall purchase decisions and satisfaction of meat products. Color is the major factor affecting consumers' purchasing decisions. Tenderness is the most important palatability attribute deciding consumers' overall eating experience (Dikeman, 1987; Miller et al., 1995). Eilert (2005) reported that case-ready packaging in the meat industry was growing at a rapid rate. Case-ready packaging generally includes modified atmosphere packaging (MAP) with specific gases. There are several advantages of MAP, including use of a centralized location, improved control of sanitation, more consistent products, and increased marketing flexibility (Jeyamkóndan et al., 2000; Kropf, 2004). Packaging beef in high-oxygen (HiO_2) MAP results in a desirable bright red color (Behrends et al., 2003; Seyfert et al., 2005) but may have detrimental effects on other quality traits, including increased off-flavors and decreased tenderness (Tørngren, 2003; Sørheim et al., 2004; Clausen, 2004; Madsen and Clausen, 2006), as well as bone discoloration (Grobbel et al., 2006). The use of CO has been approved in the US for levels up to 0.4% in retail MAP (USFDA, 2004). Products in MAP that include CO have improved beef color stability and extended display time (Luño et al., 1998; Sørheim et al., 1999; Hunt et al., 2004).

Premature browning, originally found in ground beef, results when meat is cooked to temperatures lower than what is necessary to kill harmful pathogens but appears well done internally (Hague et al., 1994; Warren et al., 1996; and Hunt et al., 1999). This phenomenon is also found in whole muscle steaks and can be attributed to packaging environments, including HiO_2 MAP (Seyfert et al., 2004; John et al., 2005). Therefore, the objectives of this study were to evaluate the effects of different gas compositions in MAP and packaging type (MAP vs. vacuum packaging) on beef tenderness, fresh color stability, and internal cooked color.

MATERIALS AND METHODS

Samples and packaging

Paired beef longissimus lumborum muscles (n=14 pairs) from USDA Select, A-maturity carcasses were obtained from a commercial abattoir at 2 d postmortem and stored in vacuum at 2°C until 7 d postmortem. Loins from different sides were assigned to either 14 d tenderness measurement or display and then 18 d (HiO_2 MAP) or 28 d (VP and ULO_2 plus CO MAP blends)

tenderness measurement. These times were different because steaks packaged in HiO_2 MAP have a shorter shelf-life and were held for less time in dark storage. Steaks were fabricated into 2.54-cm thick steaks and assigned to initial tenderness or packaging treatments of: 1) vacuum packaging (VP; 62.2 cm Hg vac; Multivac C500; Multivac Inc., Kansas City, MO); 2) ultra-low oxygen modified atmosphere packaging (MAP) with CO (ULO_2CO) (64.6% N_2, 35% CO_2, 0.4% CO); 3) high-oxygen MAP (HiO_2) (80% O_2, 20% CO_2); 4) 99.6% CO_2, 0.4% CO (ULO_2COCO_2); 5) 99.6% N_2, 0.4% CO (ULO_2CON_2); (AirGas certified gas, MidSouth, Inc., Tulsa, OK); or 6) 99.6% Ar, 0.4% CO (ULO_2COAr; Linweld certified gas, Linweld, Manhattan, KS) and subsequent color and tenderness measurements. Different packaging treatment gas blends were chosen to evaluate the effects of single gases commonly used in MAP on color and tenderness and included 0.4% CO to allow steaks to be red in color. The use of argon, an inert gas, is not currently approved for use with MAP in the US, but was evaluated to determine if it had any positive or negative effects on color or tenderness. Steaks packaged in MAP (Ross Jr. S-3180, Ross, Midland, VA) were placed in 4.32 cm deep rigid plastic trays (CS977, Cryovac Sealed Air Corp., Duncan, SC) and covered with oxygen-barrier film (Lid 550; 1.0 mils; less than 20.0 oxygen transmission cc/24 h/m^2 at 4.4°C with 100% relative humidity (RH); and moisture vapor transmission less than 0.1 g/24 h/645.2 cm^2 at 4.4°C and 100% RH; Cryovac Sealed Air Corp., Duncan, SC). An additional 3 steaks were cut posterior to the first 7 steaks, cut in half (dorsal to ventral), randomly assigned to a packaging treatment, stored at 2°C in dark storage until 14 d postmortem, and used for cooked internal color. High-oxygen MAP was held in dark storage (2°C) for 4 days and then put into simulated retail display and removed on d 18 postmortem. All packaging treatments without O_2 were held in dark storage (2°C) for 14 days and then put into simulated retail display and removed on d 28 postmortem. Steaks in all packaging treatments used for 14 d postmortem WBSF were held for 7 d in the dark and then cooked for WBSF measurement. Dark storage times for HiO_2 and ULO_2 MAP were developed to mimic what would happen in industry. An activated oxygen scavenger (ActiveTech™; Pactiv, Chicago, IL) was included in each of the ULO_2 packages to eliminate any residual O_2. One steak from each loin was vacuum packaged and used for initial WBSF on d 7 postmortem.

Cooking of steaks

Steaks for Warner-Bratzler shear force (WBSF) or internal cooked color were cooked in a forced-air convection oven (Blodgett, model DFG-102 CH3, G.S. Blodgett Co., Burlington, VT) set at 163°C. Steaks were turned at an internal temperature of 40°C and cooked to an internal temperature of 70°C, as monitored with copper-constantan thermocouples in the approximate geometric center of each steak.

Warner-Bratzler shear force

On d 7 postmortem, d 14 postmortem, and at the end of each display (d 18 for HiO_2 MAP or d 28 for VP and ULO_2 plus CO MAP postmortem), steaks from all packaging treatments were cooked, cooled to room temperature, and stored at 2°C overnight. Eight 1.27 cm cores were removed parallel to the muscle fibers using a 1.27 cm corer (G-R Manufacturing Co., Manhattan, KS) attached to an electric drill (Craftsman 3/8" Electric Drill, Sears, Hoffman Estates, IL). Cores were then sheared once perpendicular to the muscle fibers using a Warner-Bratzler V-shaped blunt blade (G-R Manufacturing Co., Manhattan, KS) attached to a 50 kg load cell of an Instron Universal Testing Machine (model 4201, Instron Corp., Canton, MA) with a crosshead speed of 250 mm/min. Peak shear force was recorded in kg and values from the 8 cores were averaged.

pH

The pH of steaks was measured on d 14 postmortem by inserting the tip of pH probe (MPI pH probe, glass electrode, Meat Probes Inc., Topeka, KS) into the longissimus lumborum muscle.

Display case

Packages were displayed (Unit model DMF8, Tyler Refrigeration Corp., Niles, MI) under continuous fluorescent lighting (2153 lux, 3000 K and CRI = 85, Bulb model F32T8/ADV830/Alto, Philips, Bloomfield, NJ) for 7 d at 2°C. Packages were rotated twice daily in order to maintain a random sample placement.

Color Measurements

Trained visual color panelists (n=10) evaluated initial color on d 0 of display and display color and surface discoloration on d 0 to 7 of display, once each day. Initial color was determined using the following scale: 1) purplish red or reddish tan of vacuum package, 2) bleached, pale red, 3) slightly cherry red, 4) moderately light cherry red, 5) cherry red, 6) slightly dark red, 7) moderately dark red, 8) dark red, 9) very dark red. The color scale used by panelists for steaks packaged in MAP was: 1) very bright red or very bright pinkish red, 2) bright red or bright pinkish red, 3) dull red or dull pinkish red, 4) slightly dark red or slightly dark pinkish red, 5) reddish tan or pinkish tan, 6) moderately dark red or reddish tan or moderately dark pinkish red or pinkish tan, 7) tannish red or tannish pink, 8) tan to brown. Steaks packaged in VP were evaluated with the following scale: 1) very bright purplish red or very bright purplish pink, 2) bright purplish red or bright purplish pink, 3) dull purplish red or dull purplish pink, 4) slightly dark purplish red or slightly dark purplish pink, 5) purplish tan or pinkish tan, 6) moderately dark purplish red or moderately dark purplish pink, 7) tannish purple red or tannish purple pink, and 8) tan to brown. For all steaks, discoloration was considered as a percentage of surface metmyoglobin and the following scale was used to evaluate this: 1) none (0%), 2) slight discoloration (1-19%), 3) small discoloration (20-39%), 4) modest discoloration (40-59%), 5) moderate discoloration (60-79%), 6) extensive discoloration (80-99%), and 7) total discoloration (100%). Color scales were used to half-point increments, whereas discoloration was scored to whole-point increments.

Instrumental color (L*, a*, and b*) was measured using a HunterLab MiniScan™ XE Plus Spectrophotometer (Model 45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA) on d 0 and d 7 of display for all packaging treatments. To accomplish reading instrumental color on MAP steaks, the half steaks from steaks 8-10 were packaged and stored in dark storage until d 0 of their display counterpart steaks. Steaks were scanned immediately after opening packages. Each steak was scanned in triplicate and values averaged.

Cooked internal color was evaluated on the half steaks used for instrumental color on d 0 of display. Steaks were allowed to cool briefly after cooking before being sliced in half and instrumental color (L*, a*, b*) measured using a HunterLab MiniScan™ XE Plus Spectrophotometer immediately after slicing. The internal surface was scanned in triplicate and

values averaged. Hue angle was calculated using the $\tan^{-1}$ b*/a* and saturation index was calculated using $(a^{*2} + b^{*2})^{1/2}$ (Hunt et al., 1991).

Statistical Analysis

The experimental design was a split-plot design with the whole plot being a randomized complete block design, with block being animal. The subplot consisted of steaks from each loin. The MIXED procedure of SAS was used to analyze the data. For WBSF, the fixed effects were comparison of the mean of d 7 to the mean of all other data, packaging treatment(comparison of the mean of d 7 to the mean of all other data), day(comparison of the mean of d 7 to the mean of all other data), and day × packaging treatment(comparison of the mean of d 7 to the mean of all other data). Instrumental color, visual color, and discoloration fixed effects were packaging treatment, day, and packaging treatment × day. The fixed effect for initial color and cooked color was packaging treatment. Random effects for WBSF, instrumental color, initial color and cooked color included animal and side(animal). Random effects for visual color and discoloration were animal, side(animal), and packaging treatment × side(animal), with day serving as a repeated measure. Means were separated using Fisher's Protected LSD with Prasad-Rao-Jeske-Kackar-Harville standard errors and the Kenward-Roger degrees of freedom. Highest order interactions were reported when they were significant or main effects when no interactions were significant. Significance was determined at probability values of $P < 0.05$.

RESULTS AND DISCUSSION

Warner-Bratzler shear force

There was a packaging treatment × d interaction ($P < 0.004$) for WBSF (Table 2.1, Appendix A – Figure A.1). Warner-Bratzler shear force (WBSF) values from longissimus lumborum steaks indicate that, as a system, HiO_2 MAP (d 18 postmortem) resulted in steaks being less tender than those packaged in ULO_2 with CO MAP or VP (d 28 postmortem). There were no differences ($P > 0.05$) in WBSF on d 14 postmortem and all treatments were more tender ($P < 0.01$) on d 14 postmortem than d 7 postmortem. Conversely, steaks packaged in HiO_2 MAP were less tender ($P < 0.05$) than other treatments at the end of display, likely due to 10 d less aging time (d 18 vs. 28 postmortem) because of a shorter dark storage period (4 d) for HiO_2 MAP than ULO_2CO MAP and VP packaging treatments (14 d). Steaks packaged in all

packaging treatments used for 14 d postmortem WBSF were held for 7 d in the dark and then cooked for WBSF measurement. Dark storage times for HiO_2 and ULO_2 atmospheres were developed to mimic what would happen in industry. There was a trend ($P = 0.06$) for steaks packaged in VP to be more tender than steaks packaged in ULO_2CO MAP on d 28 postmortem.

The results of other studies show more distinct differences in tenderness due to packaging environment. Steaks packaged in HiO_2 MAP have been shown to be less tender after 7 to 14 d than steaks packaged in VP or ULO_2 with or without CO MAP by instrumental and/or trained sensory panelists (Tørngren, 2003; Sørheim et al., 2004; Clausen, 2004; and Madsen and Clausen, 2006). These studies used steaks from heifers, cows, or bulls and were most likely fed different types of diets than the traditional grain-fed diets most often used in U.S. cattle harvested, such as the A-maturity, USDA Select carcasses obtained in this study. The differences in cattle gender, age, and feeding regimens between this study and others may have played a role in the results I saw compared to the other studies.

Table 2.1 Warner-Bratzler shear force (WBSF) packaging treatment $\times$ d means and SE for longissimus lumborum steaks packaged in different atmospheres

Treatment[a]	WBSF (kg)					
	d postmortem					
	7[b]	SE	14	SE	18 or 28[c]	SE
ULO_2COAr	5.32[d]	0.14	4.93[e]	0.17	3.88[f]	0.17
ULO_2COCO_2	5.32[d]	0.14	4.77[e]	0.17	3.84[f]	0.17
HiO_2	5.32[d]	0.14	4.83[e]	0.17	4.63[e]	0.17
ULO_2CO	5.32[d]	0.14	4.75[e]	0.17	4.01[f]	0.17
ULO_2CON_2	5.32[d]	0.14	4.85[e]	0.17	3.87[f]	0.17
VP	5.32[d]	0.14	4.77[e]	0.17	3.68[f]	0.17

[a]ULO_2COAr (99.6% Ar, 0.4% CO); ULO_2COCO_2 (99.6% CO_2, 0.4% CO); HiO_2 (80% O_2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); ULO_2CON_2 (99.6% N_2, 0.4% CO); and VP (vacuum packaging)
[b]d 7 postmortem is d 0 of packaging
[c]d 18 postmortem for the HiO_2 treatment and d 28 postmortem for the ULO_2 and VP treatments
[def]Means with different superscript letters differ ($P < 0.01$)

pH

There were no ($P > 0.05$) differences in pH for longissimus lumborum steaks packaged in different packaging treatments (Table 2.2). These results agreed with expected results for pH, as packaging treatment should not alter muscle pH.

Table 2.2 pH and initial visual color score means and SE[a] for longissimus lumborum steaks packaged in different atmospheres

		Initial color[b]
Treatment[c]	pH	d 0 of display
ULO$_2$COAr	5.6	5.3[f]
ULO$_2$COCO$_2$	5.5	5.1[ef]
HiO$_2$	5.5	4.9[e]
ULO$_2$CO	5.5	5.2[f]
ULO$_2$CON$_2$	5.6	5.4[f]
VP	5.5	1.0[d]

[a]SE=0.02 for pH; SE=0.13 for initial color
[b]1=purplish red or reddish tan of vacuum package, 4=moderately light cherry red, 5=cherry red, 6=slightly dark red
[c]ULO$_2$COAr (99.6% Ar, 0.4% CO); ULO$_2$COCO$_2$ (99.6% CO$_2$, 0.4% CO); HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); ULO$_2$CON$_2$ (99.6% N$_2$, 0.4% CO); and VP (vacuum packaging)
[def]Means with different superscript letters differ ($P < 0.05$)

Display color and discoloration

Initial color was evaluated to characterize steak color at the time they were put into the display case on d 0 of display. There was a main effect of packaging treatment ($P < 0.001$) for initial color as expected. Steaks packaged in VP had the typical purplish red color and all other treatments were classified around cherry red, with a few minor statistical differences among them (Table 2.2). Steaks packaged in HiO$_2$ and ULO$_2$COCO$_2$ MAP had the same ($P > 0.05$) initial color. Steaks packaged in ULO$_2$COAr, ULO$_2$COCO$_2$, ULO$_2$CO, and ULO$_2$CON$_2$ MAP were similar ($P > 0.05$) in initial color. Including CO or O$_2$ in the MAP allows meat to be cherry red in color, but excluding O$_2$ from the package, as done in VP, results in meat being a purplish red color.

There was a packaging treatment × d interaction ($P < 0.001$) for display visual color (Table 2.3; Appendix A – Figure A.2). Display color scores indicated that steaks from all treatments became darker ($P < 0.05$) as d of display increased, as was expected. Steaks packaged in HiO$_2$ MAP were slightly brighter ($P < 0.05$) according to display color score than steaks packaged in ULO$_2$COAr or ULO$_2$CO MAP on d 0 of display. Vacuum packaged steaks were the most consistent in display color throughout the 7 d of display, and only changed from bright purplish red or pink to dull purplish red or pink for the entire display period. Steaks in VP

were expected to be stable in color and not change much throughout the 7 d of display; however, many consumers find the purplish red color of VP meat undesirable regardless of the consistent color in display. Steaks packaged in HiO_2 MAP were an undesirable reddish tan by d 7 of display, whereas steaks packaged in the ULO_2CO MAP treatments were either dull red or slightly dark red by d 7 of display.

Table 2.3 Display color score packaging treatment × d means and SE[a] for longissimus lumborum steaks packaged in different atmospheres and displayed

	Visual color							
	d of display							
Treatment[b]	0	1	2	3	4	5	6	7
ULO_2COAr[c]	$3.1^{e,z}$	$3.2^{e,yz}$	$3.4^{f,z}$	$4.0^{g,z}$	$4.0^{g,z}$	$4.2^{h,z}$	$4.4^{i,yz}$	$4.5^{i,y}$
ULO_2COCO_2[c]	$2.9^{e,yz}$	$3.1^{e,yz}$	$3.2^{f,yz}$	$3.3^{fg,y}$	$3.5^{g,xy}$	$3.7^{h,xy}$	$3.8^{hi,x}$	$3.8^{i,w}$
HiO_2[c]	$2.7^{e,y}$	$2.9^{f,xy}$	$3.1^{g,xy}$	$3.4^{h,y}$	$3.8^{i,z}$	$4.2^{j,z}$	$4.4^{k,yz}$	$5.0^{l,z}$
ULO_2CO[c]	$3.0^{e,z}$	$3.0^{e,yz}$	$3.2^{f,xyz}$	$3.6^{g,y}$	$3.7^{h,yz}$	$4.0^{i,yz}$	$4.2^{j,y}$	$4.2^{j,x}$
ULO_2CON_2[c]	$2.9^{e,yz}$	$3.3^{f,z}$	$3.4^{g,z}$	$3.9^{h,z}$	$4.0^{h,z}$	$4.3^{i,z}$	$4.5^{j,z}$	$4.4^{i,xy}$
VP[d]	$2.9^{f,yz}$	$2.7^{e,x}$	$2.9^{f,x}$	$3.0^{f,x}$	$3.3^{g,x}$	$3.7^{i,x}$	$3.5^{h,x}$	$3.3^{g,v}$

[a]SE=0.15
[b]ULO_2COAr (99.6% Ar, 0.4% CO); ULO_2COCO_2 (99.6% CO_2, 0.4% CO); HiO_2 (80% O_2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); ULO_2CON_2 (99.6% N_2, 0.4% CO); and VP (vacuum packaging)
[c]2=bright red or bright pinkish red, 3=dull red or dull pinkish red, 4=slightly dark red or slightly dark pinkish red, 5=reddish tan or pinkish tan, 6=moderately dark red or reddish tan or moderately dark pinkish red or pinkish tan
[d]2=bright purplish red or bright purplish pink, 3=dull purplish red or dull purplish pink, 4=slightly dark purplish red or slightly dark purplish pink, 5=purplish tan or pinkish tan, 6=moderately dark purplish red or moderately dark purplish pink
[efghijkl]Means within the same treatment (row) with different superscript letters differ ($P < 0.05$)
[vwxyz]Means within the same d of display (column) with different superscript letters differ ($P < 0.05$)

There was a packaging treatment × d interaction ($P < 0.001$) for discoloration scores (Table 2.4; Appendix A – Figure A.3). Steaks packaged in VP or the four ULO_2 MAP blends with CO had little or no surface discoloration over the 7 d of display. Steaks packaged in HiO_2 MAP discolored faster ($P < 0.05$) and to a greater extent ($P < 0.05$) than those packaged in any of the ULO2 MAP or VP treatments. Steaks packaged in HiO_2 MAP discolored ($P < 0.05$) by d 4 of display and had 56% more ($P < 0.05$) metmyoglobin discoloration than those packaged in any other packaging treatment. On d 4 of display, steaks packaged in ULO_2COCO_2 and HiO_2

MAP had similar ($P < 0.05$) discoloration, but by d 5 of display steaks packaged in HiO_2 MAP had more ($P < 0.05$) discoloration than steaks in all other packaging treatments. Including O_2 in the package allows for oxidation of myoglobin and thus resulted in a reddish tan color by d 7 of display. Excluding O_2 from the package, as with VP or ULO_2CO MAP treatments, allows myoglobin to remain in a more stable form (red) longer and delayed the onset of metmyoglobin (tan/brown) color formed through the oxidation of myoglobin. Figure A.4 (Appendix A) shows differences in color on d 0, 4, and 7 of display.

Table 2.4 Discoloration score packaging treatment $\times$ d means and SE[a] for longissimus lumborum steaks packaged in different atmospheres and displayed

	Discoloration score[b]							
	d of display							
Treatment[c]	d 0	d 1	d 2	d 3	d 4	d 5	d 6	d 7
ULO_2COAr	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,y}$	$1.0^{d,y}$	$1.0^{d,y}$	$1.0^{d,y}$
ULO_2COCO_2	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,yz}$	$1.1^{d,y}$	$1.2^{e,y}$	$1.2^{e,y}$
HiO_2	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.2^{d,z}$	$1.4^{e,z}$	$1.7^{f,z}$	$2.2^{g,z}$	$2.7^{h,z}$
ULO_2CO	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,y}$	$1.0^{d,y}$	$1.1^{d,y}$	$1.0^{d,y}$
ULO_2CON_2	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,y}$	$1.1^{d,y}$	$1.1^{d,y}$	$1.0^{d,y}$
VP	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,y}$	$1.0^{d,y}$	$1.1^{d,y}$	$1.0^{d,y}$

[a]SE=0.12
[b]1=none (0%), 2=slight discoloration (1-19%), 3=small discoloration (20-39%)
[c]ULO_2COAr (99.6% Ar, 0.4% CO); ULO_2COCO_2 (99.6% CO_2, 0.4% CO); HiO_2 (80% O_2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); ULO_2CON_2 (99.6% N_2, 0.4% CO); and VP (vacuum packaging)
[defgh]Means with different superscript letters across d of display (row) differ ($P < 0.05$)
[yz]Means within the same d of display (column) with different superscript letters differ ($P < 0.05$)

Instrumental color

There was a packaging treatment $\times$ d interaction for L*, a*, b*, and saturation index values ($P < 0.001$) (Table 2.5). Steaks packaged in the four ULO_2CO MAP treatments had higher ($P < 0.05$) L* values than steaks packaged in HiO_2 MAP or VP. Steaks packaged in ULO_2CO and ULO_2CON_2 MAP were redder ($P < 0.05$) than steaks packaged in HiO_2 MAP on d 0 of display. Steaks packaged in ULO_2CO and ULO_2CON_2 MAP had no change ($P > 0.05$) in a* values, whereas steaks packaged in ULO_2COAr and ULO_2COCO_2 MAP had increased ($P < 0.05$) a* values from d 0 to d 7 of display. Steaks packaged in HiO_2 MAP had drastically lower

a* values on d 7 of display compared to d 0 of display. Vacuum packaged steaks had decreased ($P < 0.05$) a* values, although not nearly the extent of a reduction as found in steaks packaged in HiO_2 MAP.

A greater saturation index indicates a greater saturation of red (Hunt et al., 1991). Steaks packaged in HiO_2 MAP had a dramatic decrease ($P < 0.05$) in saturation index from d 0 to d 7 of display and was much lower ($P < 0.05$) than the four ULO_2CO MAP treatments by d 7 of display.

Table 2.5 Instrumental color packaging treatment × d means and SE[a] for longissimus lumborum steaks packaged in different atmospheres.

Treatment[b]	L*		a*		b*		Saturation index	
	d 0	d 7	d 0	d 7	d 0	d 7	d 0	d 7
ULO_2COAr	50.5[g]	48.3[ef]	31.1[ef]	32.8[gh]	17.4[ef]	18.8[gh]	35.6[f]	37.9[gh]
ULO_2COCO_2	50.8[g]	50.0[fg]	31.9[efg]	33.7[h]	18.5[gh]	19.4[gh]	36.9[fg]	38.9[h]
HiO_2	47.2[e]	51.2[g]	30.4[e]	23.8[d]	23.2[j]	16.5[e]	38.3[gh]	29.0[e]
ULO_2CO	49.3[f]	50.2[fg]	32.2[fgh]	32.3[fgh]	20.0[i]	18.5[gh]	38.0[gh]	37.2[fgh]
ULO_2CON_2	49.2[f]	49.0[f]	31.0[ef]	32.1[efgh]	18.0[fg]	18.1[fg]	35.8[f]	36.9[fgh]
VP	40.4[c]	42.5[d]	22.9[d]	20.6[c]	13.0[d]	10.9[c]	26.4[d]	23.3[c]

[a]SE=0.7 for L*, a*, and b*; SE = 0.03 for saturation index
[b]ULO_2COAr (99.6% Ar, 0.4% CO); ULO_2COCO_2 (99.6% CO_2, 0.4% CO); HiO_2 (80% O_2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); ULO_2CON_2 (99.6% N_2, 0.4% CO); and VP (vacuum packaging)
[cdefghij]Means within response variables with different superscript letters differ ($P < 0.05$)

In general, instrumental color agreed with display visual color results found by trained panelists. Although panelists found that steaks packaged in HiO_2 MAP (2.7) were brighter ($P < 0.05$) in color on d 0 of display than steaks packaged in ULO_2CO MAP (3.0), the difference found in display color score was minor. Instrumental a* values indicated that steaks packaged in ULO_2CO MAP (32.2) were brighter ($P < 0.05$) on d 0 of display than steaks packaged in HiO_2 MAP (30.4), the exact opposite from what panelists found, indicating the difference is not of practical significance. Both HiO_2 and all ULO_2 with CO MAP treatments resulted in an initial desirable red color. Argon, CO_2, and N_2 were compared at the 99.6% level with 0.4% CO (included to have meat in the red, carboxymyoglobin state and not in deoxymyoglobin) to determine if a single gas in the blends associated with MAP had an effect on beef color. The

small differences found in display color and instrumental color among the four blends of ULO_2 with CO MAP were of no practical significance.

Steaks packaged in VP, HiO_2 and ULO_2CO MAP had similar spectral reflectance means from 400 to 700 nm (Figure 2.1). At a specific wavelength (i.e. 525, 572, and 610), when two or more forms of myoglobin pigment are equal, they are considered to be isobestic (Hunt et al., 1991). Figure 2.1 indicates that VP, HiO_2 and ULO_2CO MAP are similar at isobestic wavelengths 525 and 572 nm on d 0 of display while HiO_2 and ULO_2CO MAP are similar at 610 nm. The data suggests that oxymyoglobin found in HiO_2 MAP and carboxymyoglobin found in ULO_2CO MAP are similar to each other.

Beef stored in ULO_2CO MAP maintained its red color whereas steaks packaged in HiO_2 MAP discolored more rapidly. Behrends et al. (2003) reported acceptable color stability of steaks packaged in HiO_2 MAP through d 5 of display. Other researchers showed increased times of storage beyond the 5 d Behrends et al. (2003) reported in HiO_2 MAP for steaks being red in color; however, some of these steaks were stored in dark storage and not displayed under lights as in the current study. John et al. (2005) reported that steaks in HiO_2 MAP were red in color through 14 d of storage and steaks in ULO_2CO MAP were red through 21 d of storage. They also found that the majority of steaks stored in VP remained purple in color through d 21 of storage; however, some of their VP steaks must have had residual oxygen in the package because they turned brown in color. In addition, Sørheim et al. (1999) reported bright red color and high a* values of steaks stored in ULO_2CO MAP through their 11 d of storage, whereas steaks in HiO_2 MAP discolored from d 3 to 8 of storage and VP steaks did not change in color throughout the 11 d storage period.

Figure 2.1 Reflectance means for steaks packaged in high-oxygen (HiO$_2$) and ultra-low oxygen with CO (ULO$_2$CO) modified atmosphere packaging and vacuum packaging (VP) on d 0 and d 7 of display
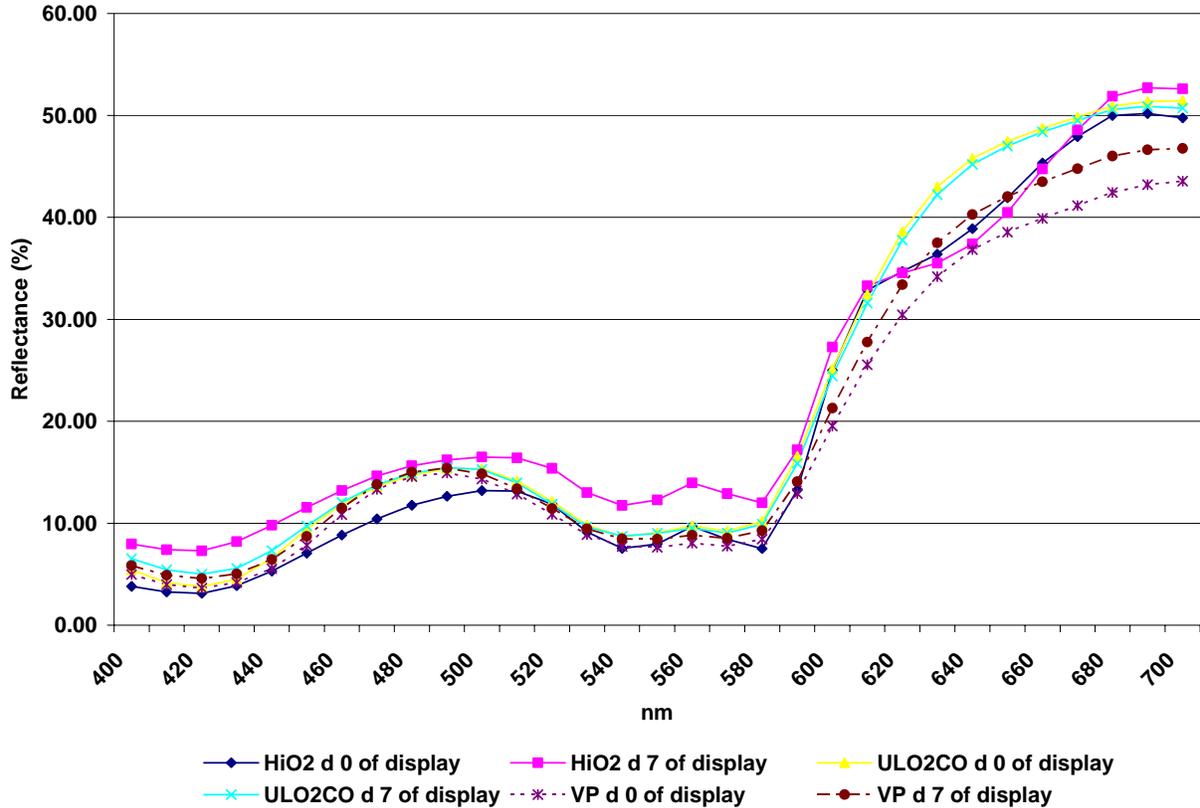
Internal cooked color

There was a packaging treatment main effect ($P < 0.001$) for internal cooked a*, b*, a*/b*, hue angle, saturation, and the ratio of 630/580 nm values (Table 2.6). L* value main effects were not significant ($P = 0.059$; data not shown). Steaks packaged in HiO$_2$ had the lowest ($P < 0.05$) a* values (brownest) for internal cooked color of all packaging treatments. Steaks packaged in ULO$_2$COCO$_2$ and in vacuum had intermediate a* values while those packaged in ULO$_2$COAr, ULO$_2$CO, and ULO$_2$CON$_2$ had the highest ($P < 0.05$) a* values (reddest). Oxidation state of myoglobin prior to cooking can alter the amount of myoglobin that is denatured, with deoxymyoglobin (purple) being the most stable followed by oxymyoglobin (red) being intermediate and metmyoglobin (brown) being least stable (Machlik, 1965). Premature browning is defined by internal cooked color of meat that is brown at temperatures where it should still appear red in color and is related to the oxidative state of meat prior to

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cooking (Warren et al., 1996). Visual and instrumental internal color of premature brown ground beef patties at 55°C was similar in visual and instrumental internal cooked color to normal ground beef patties at 75°C (Warren et al., 1996). Lavelle et al. (1995) evaluated ground beef from steers fed vitamin E supplementation and found that premature browning was not induced or prevented by vitamin E supplementation. They also reported that patties from supplemented steers cooked from the oxidative state would result in premature browning as patties from non-supplemented steers would. Results from my study agree with results of Seyfert et al. (2004) and John et al. (2005) indicating that steaks packaged in HiO$_2$ MAP prematurely turn brown in internal cooked color. Beef in the carboxymyoglobin state (MAP with CO) had less denaturation (49%) than beef in the deoxymyoglobin state (58%) when cooked to 71.1°C (Ballard, 2004). Furthermore, carboxymyoglobin has been shown to be more heat stable than oxymyoglobin (John et al., 2004). Thus, the addition of CO to MAP may increase persistent pinking (Mancini and Hunt, 2005) and could be caused by a heat-denatured CO-hemochrome instead of undenatured carboxymyoglobin (John et al., 2004). Results from my study indicated that steaks packaged in ULO$_2$COAr, ULO$_2$CO, and ULO$_2$CON$_2$ MAP had a redder ($P < 0.05$) internal cooked color than steaks packaged in VP. Steaks were cooked to a medium degree of doneness (70°C), which should result in a pinkish internal color. Steaks packaged in HiO$_2$ MAP were brown inside at this temperature. This could pose a definite safety risk, especially if consumers cook intact steaks to an internal color and do not use a meat thermometer to determine a safe endpoint cook temperature.

Table 2.6 Instrumental internal cooked color means and SE for longissimus lumborum steaks packaged in different atmospheres

| | Internal cooked color | | | | |
| | | | | | Saturation |
Treatment[a]	a*[b]	b*[c]	a*/b*[c]	Hue angle[d]	index[e]
ULO$_2$COAr	20.44[i]	18.63[hi]	1.10[i]	42.47[g]	27.70[i]
ULO$_2$COCO$_2$	18.43[h]	18.26[hi]	1.01[h]	45.03[h]	26.03[h]
HiO$_2$	9.40[g]	13.99[g]	0.67[g]	56.37[i]	16.89[g]
ULO$_2$CO	20.73[i]	19.3[j]	1.07[hi]	43.36[gh]	28.41[i]
ULO$_2$CON$_2$	20.91[i]	18.89[ij]	1.11[i]	42.26[g]	28.24[i]
VP	18.98[h]	18.08[h]	1.05[hi]	43.98[gh]	26.29[h]

[a]ULO$_2$COAr (99.6% Ar, 0.4% CO); ULO$_2$COCO$_2$ (99.6% CO$_2$, 0.4% CO); HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); ULO$_2$CON$_2$ (99.6% N$_2$, 0.4% CO); and VP (vacuum packaging)
[b]SE=0.6
[c]SE=0.3
[d]SE=0.7
[e]SE=0.5
[ghij]Means with different superscript letters within columns (instrumental measurements) differ ($P < 0.05$)

In conclusion, results from this study indicated that steaks packaged in HiO$_2$ MAP had less color stability than all other packaging treatments evaluated because they discolored faster and to a greater extent. Ultra-low oxygen + CO MAP and VP treatments had better fresh color stability than steaks packaged in HiO$_2$ MAP and had equal or better tenderness. Packaging atmospheres altered internal cooked color, with steaks packaged in HiO$_2$ MAP exhibiting premature browning. Longissimus lumborum steaks packaged in the HiO$_2$ MAP system were less tender at the end of display than other packaging treatments, which may have been because of the shorter aging time associated with the HiO$_2$ MAP system. Packaging beef in ULO$_2$CO MAP provides beef with a bright red color with extended color stability and provides for a longer aging time and increased tenderness while resulting in an internal cooked color that is expected for medium degree of doneness, both of which would be beneficial to the meat industry.

LITERATURE CITED

Ballard, C. 2004. Thermal denaturation of carboxymyoglobin and the affect of carbon monoxide and injection enhancement on display and cooked color. Masters Thesis. Kansas State University, Manhattan.

Behrends, J.M., W.B. Mikel, C.L. Armstrong, and M.C. Newman. 2003. Color stability of semitendinosus, semimembranosus, and biceps femoris steaks packaged in a high-oxygen modified atmosphere. J. Anim. Sci. 81:2230-2238.

Clausen, I. 2004. Sensory evaluation of beef loin steaks stored in different atmospheres. Proc. 50th Int. Congr. Meat Sci. Technol. Helsinki, Finland.

Dikeman, M. E. 1987. Fat reduction in animals and the effects on palatability and consumer acceptance of meat products. Proc. Meat Conf. 40:93.

Eilert., S. J. 2005. Review: New packaging technologies for the 21st century. Meat Sci. 71:122-127.

Grobbel, J. P., M. E. Dikeman, E. J. Yancey, J. S. Smith, D. H. Kropf, and G. A. Milliken. 2006. Effects of ascorbic acid, rosemary, and Origanox™ in preventing bone marrow discoloration in beef lumbar vertebrae in aerobic and anaerobic packaging systems. Meat Sci. 72(1):47-56.

Hague, M. A., K. E. Warren, M. C. Hunt, D. H. Kropf, C. L. Kastner, S. L. Stroda, and D. E. Johnson. 1994. Endpoint temperature, internal cooked color, and expressible juice color relationships in ground beef patties. J. Food Sci. 58:465-470.

Hunt, M. C., J. C. Acton, R. C. Benedict, C. R. Calkins, D. P. Cornforth, L. E. Jeremiah, D. G. Olson, C. P. Salm, J. W. Savell, and S. D. Shivas. 1991. Guidelines for meat color evaluation. Appendix to Proc. Recip. Meat Conf. 44:1-17.

Hunt, M. C., R. A. Mancini, K. A. Hachmeister, D. H. Kropf, M. Merriman, G. DelDuca, and G. Milliken. 2004. Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorganisms of beef steaks and ground beef. J. Food Sci. 69(1):C45-C52.

Hunt, M. C., O. Sørheim, and E. Slinde. 1999. Color and heat denaturation of myoglobin forms in ground beef. J. Food Sci. 64(5):847-851.

Jeyamkóndan, S., D. S. Jayas, and R. A. Holley. 2000. Review of centralized packaging systems for distribution of retail-ready meat. J. Food Prot. 63:796-804.

John, L., D. Cornforth, C. E. Carpenter, O. Sørheim, B. C. Pettee, and D. R. Whittier. 2004. Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. J. Food Sci. 69(8):C608-C614.

John, L., D. Cornforth, C.E. Carpenter, O. Sorheim, B.C. Pettee, and D.R. Whittier. 2005. Color and thiobarbituric acid values of cooked top sirloin steaks packaged in modified atmospheres of 80% oxygen, or 0.4% carbon monoxide, or vacuum. Meat Sci. 69:441-449.

Kropf, D. H. 2004. Packaging. In: W. K. Jensen, C. Devine and M. E. Dikeman (eds.) Encyclopedia of meat sciences. p. 943-969. Elsevier Ltd., Oxford.

Lavelle, C. L., M. C. Hunt, and D. H. Kropf. 1995. Display life and internal cooked color of ground beef from vitamin E-supplemented steers. J. Food Sci. 60(6):1175-1178, 1196.

Luño, M., J. A. Beltrán, and P. Roncalés. 1998. Shelf-life extension and colour stabilization of beef packaged in a low O2 atmosphere containing CO: loin steaks and ground meat. Meat Sci. 48(1/2):75-84.

Machlik, S. M. 1965. The effect of heat on bovine myoglobin derivatives in model systems and in beef semitendinosus muscle. Ph.D. Dissertation, Purdue University, West Lafayette, IN.

Madsen, N. T., and I. Clausen. 2006. Sensory evaluation of beef steaks stored in MA combinations of CO, CO2, N2, and O2. Page 405-406 in Proc 52nd Int. Congr. Meat Sci. Technol., Dublin, Ireland.

Mancini, R. A., and M. C. Hunt. 2005. Review: Current research in meat color. Meat Sci. 71:100-121.

Miller, M. F., K. L. Huffman, S. Y. Gilbert, L. L. Hammon, and C. B. Ramsey. 1995. Retail consumer acceptance of beef tenderized with calcium chloride. J. Anim. Sci. 73:2308.

Seyfert, M., M. C. Hunt, R. A. Mancini, D. H. Kropf, and S. L. Stroda. 2004. Internal premature browning in cooked steaks from enhanced beef round muscles packaged in high-oxygen and ultra-low oxygen modified atmospheres. J. Food Sci. 69(2):FCT142-FCT146.

Seyfert, M., M. C. Hunt, R. A. Mancini, K. A. Hachmeister, D. H. Kropf, J. A. Unruh, and T. M. Loughin. 2005. Beef quadriceps hot boning and modified atmosphere packaging influence properties of injection-enhanced beef round muscles. J. Anim. Sci. 83:686-693.

Sørheim, O., H. Nissen, and T. Nesbakken. 1999. The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat Sci. 52:157-164.

Sørheim, O., M. Wahlgren, B. N. Nilsen, and P. Lea. 2004. Effects of high oxygen packaging on tenderness and quality characteristics of beef longissimus muscles. Proc. 50th Int. Congr. Meat Sci. Technol., Helsinki, Finland.

Tørngren, M. A. 2003. Effect of packing method on colour and eating quality of beef loin steaks. Pages 495-496 in Proc. 49th Int. Congr. Meat Sci. Technol., Campinas, Brazil.

U. S. Food and Drug Administration (USFDA). 2004. GRAS notice number GRN 000143. Washington, D.C.:

Warren, K. E., M. C. Hunt, and D. H. Kropf. 1996. Myoglobin oxidative state affects internal cooked color development in ground beef patties. J. Food Sci. 61(3):513-515, 519.

CHAPTER 3 - Effects of Different Packaging Atmospheres and Injection-Enhancement on Beef Tenderness, Sensory Attributes, Desmin Degradation, and Display Color

ABSTRACT

The objectives of the study were to determine the effects of packaging atmosphere and injection-enhancement on tenderness, sensory traits, desmin degradation, and display color of different beef muscles. Longissimus lumborum (LL; n=12 pairs); semitendinosus (ST; n=12 pairs); and triceps brachii (TB; n=24 pairs – 12 from the same carcass as the LL and ST and 12 additional pairs) were obtained from the same USDA Select, A-maturity carcasses. On d 7 postmortem, each muscle from one side of the carcass was injection-enhanced and muscles from the other side were non-enhanced. Steaks 2.54 cm-thick were cut from the muscles and packaged in vacuum packaging (VP); ultra-low oxygen with CO (ULO_2CO) (0.4% CO/35% CO_2/69.6% N_2) modified atmosphere packaging (MAP); or high-oxygen MAP (HiO_2) (80% O_2/20% CO_2) and assigned to either 14 d tenderness measurement or display followed by 18 or 28 d tenderness measurement. Steaks packaged in HiO_2 MAP were in dark storage (2°C) for 4 d and all other steaks for 14 d. Steaks for Warner-Bratzler shear force (WBSF), sensory panel (n=8 trained panelists), and desmin degradation were cooked to 70°C. Steaks were displayed under fluorescent lighting (2153 lux, 3000 K) for 7 d. Trained color panelists (n=10) assigned display color scores. Enhanced steaks had lower ($P < 0.05$) WBSF values than non-enhanced steaks. Sensory panelists found that non-enhanced steaks packaged in ULO_2CO MAP or VP were more tender ($P < 0.05$), had more ($P < 0.05$) beef flavor, and had less ($P < 0.05$) off-flavors than steaks packaged in HiO_2 MAP. The LL and TB were more tender ($P < 0.05$) according to myofibrillar tenderness than the ST. Non-enhanced steaks were less ($P < 0.05$) juicy than enhanced steaks. The most common off-flavors associated with steaks packaged in HiO_2 MAP were oxidative or rancid. Enhanced steaks had more ($P < 0.05$) off-flavors than non-enhanced steaks, with typical descriptors of salty, metallic or chemical, in addition to an undesirable mushy texture. Desmin degradation was not affected ($P > 0.05$) by type of packaging but increased ($P < 0.05$) from 7 to 14 d postmortem. Enhanced steaks were darker ($P < 0.05$) initially than non-enhanced steaks.

Steaks packaged in HiO_2 MAP discolored at a faster rate ($P < 0.05$) and to a greater extent ($P < 0.05$) than steaks packaged in VP or ULO_2CO MAP. Non-enhanced muscles packaged in VP and ULO_2CO MAP resulted in stable display color, very desirable tenderness and flavor compared to those packaged in HiO_2 MAP.

Key Words: beef, display color, injection-enhancement, modified atmosphere packaging, postmortem proteolysis, sensory attributes

INTRODUCTION

Case-ready meat provides many benefits, including both quality and safety. Packaging meat in high-oxygen (HiO$_2$) modified atmosphere packaging (MAP) results in a desirable bright red display color (Behrends et al., 2003; Seyfert et al., 2005) but may have increased off-flavors and decreased tenderness. Steaks aged and packaged in HiO$_2$ MAP had more off-flavor, including warmed-over flavor, and were less tender and juicy than steaks aged in vacuum packaging (VP) (Tørngren, 2003; Sørheim et al., 2004; Clausen, 2004; and Madsen and Clausen, 2006). Seyfert et al. (2005) found that beef quadriceps injection-enhanced and packaged in HiO$_2$ MAP were tougher and had more off-flavors than those in ultra-low oxygen MAP. Clausen (2004) believes that the detrimental effects of O$_2$ on tenderness may be caused by protein oxidation. Rowe et al. (2004) found that oxidation of beef muscle proteins early postmortem inactivated μ-calpain and decreased myofibrillar proteolysis, thus potentially limiting tenderization.

Injection-enhancement improves tenderness and juiciness while decreasing variation and is used in conjunction with MAP. Enhancement of beef resulted in more tender and juicy steaks than non-enhanced steaks (Vote et al., 2000; Lawrence 2003a; Wicklund et al., 2005; Hoffman, 2006). Several researchers have found an increase in beef flavor associated with enhanced steaks (Vote et al., 2000; Knock et al., 2006a), whereas others have reported a decreased or no change in beef flavor (Robbins et al., 2003; Molina et al., 2005; Hoffman, 2006; Stetzer et al., 2007). Off-flavors have been associated with enhanced beef, including salty and oxidative (Seyfert et al., 2005; Knock et al., 2006a). The objectives of the study were to determine the effects of packaging atmosphere and injection-enhancement on beef longissimus lumborum, semitendinosus, and triceps brachii tenderness, sensory traits, desmin degradation, and display color.

MATERIALS AND METHODS

Samples, packaging, and injection-enhancement

Paired longissimus lumborum (n=12 pairs); semitendinosus (n=12 pairs); and triceps brachii (n=24 pairs – 12 from same carcass as the longissimus lumborum and semitendinosus and 12 additional pairs) from the same USDA Select, A-maturity carcasses were obtained at a

commercial abattoir 2 d postmortem. On d 7 postmortem, each muscle from one side of the carcass was enhanced with beef broth, potassium lactate, sodium phosphate, salt, and natural flavoring (rosemary) solution (proprietary formulation) and the muscles from the opposite side were non-enhanced. Steaks 2.54 cm thick were cut from the muscles and packaged in treatments of 1) vacuum packaging (VP; 62.2 cm Hg vac; Multivac C500; Multivac Inc., Kansas City, MO); 2) ultra-low oxygen modified atmosphere packaging (MAP) with CO (ULO$_2$CO) (64.6% N$_2$, 35% CO$_2$, 0.4% CO); or 3) high-oxygen MAP (HiO$_2$) (80% O$_2$, 20% CO$_2$) (AirGas certified gas, MidSouth, Inc., Tulsa, OK) and assigned to either 14 d tenderness measurement or to display followed by 18 or 28 d tenderness measurement. Steaks packaged in MAP (Ross Jr. S-3180, Ross, Midland, VA) were packaged in 4.32 cm deep rigid plastic trays (CS977, Cryovac Sealed Air Corp., Duncan, SC) and covered with oxygen-barrier film (Lid 550; 1.0 mils; less than 20.0 oxygen transmission cc/24 h/m^2 at 4.4°C with 100% relative humidity (RH); and moisture vapor transmission less than 0.1 g/24 h/645.2 cm^2 at 4.4°C and 100% RH; Cryovac Sealed Air Corp., Duncan, SC). High-oxygen MAP was held in dark storage (2°C) for 4 d and then put into simulated retail display and removed on d 18 postmortem. All packaging treatments without O$_2$ were held in dark storage (2°C) for 14 d and then put into simulated retail display and removed on d 28 postmortem. Steaks packaged in all packaging treatments used for 14 d postmortem WBSF were held for 7 d in the dark after packaging and then cooked for WBSF measurement. Dark storage times were developed to mimic what would happen in industry. An activated oxygen scavenger (ActiveTech™; Pactiv, Chicago, IL) was included in each of the ULO$_2$ packages to eliminate any residual O$_2$. One steak from each loin was vacuum packaged and used for initial WBSF on d 7 postmortem.

pH

The pH of steaks was measured on d 14 postmortem by inserting the tip of pH probe (MPI pH probe, glass electrode, Meat Probes Inc., Topeka, KS) into 1 location of each muscle.

Cooking of steaks

Steaks for Warner-Bratzler shear force (WBSF) or internal cooked color were cooked in a forced-air convection oven (Blodgett, model DFG-102 CH3, G.S. Blodgett Co., Burlington, VT) set at 163°C. Steaks were turned at an internal temperature of 40°C and cooked to an

internal temperature of 70°C, as monitored with copper-constantan thermocouples in the approximate geometric center of each steak.

Warner-Bratzler shear force

On d 7 postmortem, d 14 postmortem, and at the end of each display (d 18 or 28 postmortem), steaks from all packaging treatments were cooked, cooled to room temperature, and stored at 2°C overnight. Eight 1.27 cm cores were removed parallel to the muscle fibers using a 1.27 cm corer (G-R Manufacturing Co., Manhattan, KS) attached to an electric drill (Craftsman 3/8" Electric Drill, Sears, Hoffman Estates, IL). Cores were then sheared once perpendicular to the muscle fibers using a Warner-Bratzler V-shaped blunt blade (G-R Manufacturing Co., Manhattan, KS) attached to a 50 kg load cell of an Instron Universal Testing Machine (model 4201, Instron Corp., Canton, MA) with a crosshead speed of 250 mm/min. Peak shear force was recorded in kg and values from the 8 cores were averaged.

Sensory Analysis

One steak from each muscle and each packaging atmosphere was removed from MAP on d 18, vacuum packaged, and frozen at -20°C for later sensory analysis. Steaks already packaged in VP also were frozen. Panelists (n=8) were trained according to AMSA guidelines (1995) for evaluation of steaks. Steaks were thawed overnight (2°C), cooked to 70°C, sliced into 2.54 cm × 1.27 cm × 1.27 cm samples, and served warm to panelists. Samples were kept warm in blue enamel double boiler pans with warm water in the bottom portion of the pan. Panelists evaluated samples in duplicate for myofibrillar tenderness, juiciness, beef flavor intensity, amount of connective tissue, overall tenderness, and off-flavor using an eight-point scale. The scale used for myofibrillar and overall tenderness was, 1) extremely tough, 2) very tough, 3) moderately tough, 4) slightly tough, 5) slightly tender, 6) moderately tender, 7) very tender, and 8) extremely tender. For juiciness, the scale was 1) extremely dry, 2) very dry, 3) moderately dry, 4) slightly dry, 5) slightly juicy, 6) moderately juicy, 7) very juicy, and 8) extremely juicy. The scale used for beef flavor was, 1) extremely bland, 2) very bland, 3) moderately bland, 4) slightly bland, 5) slightly intense, 6) moderately intense, 7) very intense, and 8) extremely intense. The scale used for connective tissue and off flavor intensity was, 1) abundant, 2) moderately abundant, 3) slightly abundant, 4) moderate, 5) slight, 6) traces, 7) practically none, and 8) none. Scores were given to the nearest half-point increment.

Cooking loss

Steaks used for sensory analysis were weighed prior to cooking (initial weight), allowed to cool for approximately 5 min at room temperature and weighed again (final weight). Cooking loss was calculated by [(initial weight – final weight) / initial weight]*100.

Immunoblotting

Desmin degradation was used as a measure of postmortem proteolysis. Extraction, electrophoresis, Western blotting, and quantification of desmin was measured on d 7 and 14 postmortem in conjunction with scientists at the Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE, ARS, USDA according to procedures outlined by Wheeler and Koohmaraie (1999) and Wheeler et al. (2002). Reagents were made with Sigma chemicals (Sigma-Aldrich, St. Louis, MO) and reagents used for electrophoresis were obtained from Bio-Rad Laboratories (Hercules, CA) unless otherwise stated. Cores from Warner-Bratzler shear force (WBSF) were trimmed free of the cooked crust and pulverized in liquid N_2. Muscle extracts were done by homogenizing 1 g of muscle in 10 mL of 50 mM Tris, 10mM EDTA, pH 8.3, for 20 s using a polytron on speed setting 4 (Brinkmann Instruments, Westbury, NY). Sample homogenate (0.5 mL) and 2× treatment buffer (0.5 mL; 0.125 M tris, 4% sodium dodecyl sulfate (SDS), and 20% glycerol; pH 6.8) were vortexed and incubated in a 50°C waterbath for 20 min, mixed with repeated pipetting, and heated for an additional 5 min. Samples were then centrifuged (Eppendorf 5414 C, Eppendorf AG, Hamburg, Germany) for 20 min to pellet insoluble material.

Protein concentration was determined using the micro-BCA protein assay (Pierce, Rockford, IL) with a 1:5 dilution of supernatant and 1× treatment buffer in triplicate. BCA reagent (200 µl) was added to each sample, incubated at 37°C for 30 min, and read at 562 nm (SPECTRAmax Plus 384, Molecular Devices Corp., Sunnyvale, CA). Samples were diluted to 3 mg/mL using protein denaturing buffer (PDB) containing 2× treatment buffer (listed above), 10% mercaptoethanol (MCE) and 0.8% bromophenol blue. Samples were vortexed, heated at 50°C for 10 min, and frozen until used for electrophoresis.

Desmin was separated on 10% gels (1.5 M tris, pH 8.8; 30% acrylamide (37.5:1 acrylamide to bisacrylamide); 10% SDS; 10% ammonium persulfate (APS); and TEMED) with 4% stacker (0.5 M tris, pH 6.8; 30% acrylamide (37.5:1); 10% SDS; 10% APS; and TEMED) in

buffer containing 0.25 M tris and 1.92 M glycine. Samples were loaded at 15 µg of protein per lane and 0 h standards were loaded at 18 µg of protein per lane. Discontinuous gels were run at 200 V for 45 min. Gels were transferred to Hybond-P PVDF membranes (Amersham Biosciences, Piscataway, NJ) for 1 h at 200 mA in buffer containing 25 mM tris, 193 mM glycine, and 10% methanol. Membranes were blocked with 2.5% sheep serum in tris-buffered saline (20 mM tris, 137 mM NaCl, and 5 mM KCl), pH 7.4, containing 0.05% Tween-20 (TTBS) for 1 h. Membranes were incubated with gentle shaking at room temperature for 1 h with primary antibody as follows: monoclonal anti-desmin 1:300 for semitendinosus and triceps brachii or 1:100 for longissimus samples (clone D3; developed by D. A. Fischman and obtained from the Developmental Studies Hybridomal Bank). Membranes were rinsed twice and washed once for 15 min and twice for 5 min using TTBS. Bound primary antibodies were labeled (1 h at room temperature) with Immunopure goat anti-mouse IgG horseradish peroxidase conjugated secondary antibodies diluted 1:10,000 (Pierce, Rockford, IL). Membranes were washed once for 15 min and 4 times for 5 min with TTBS. Detection of antibody binding was done by incubating the membranes for 5 min using the SuperSignal West Dura Extended Duration Substrate (Pierce, Rockford, IL) and exposing the membrane for 5 min with a ChemiImager 4000 digital imaging analysis system (Alpha Innotech, San Leandro, CA). Protein bands were quantified using the ChemiImager 4000 digital imaging analysis system. Each blot contained three lanes of at-death (0 h postmortem) beef muscle specific (longissimus, semitendinosus, or triceps brachii) samples that were averaged as a reference standard. The extent of desmin degradation was determined by expressing the density of protein bands of treatments as a percentage of degradation relative to that of the reference standard within each blot.

Display case

Packages were displayed (Unit model DMF8, Tyler Refrigeration Corp., Niles, MI) under continuous fluorescent lighting (2153 lux, 3000 K and CRI = 85, Bulb model F32T8/ADV830/Alto, Philips, Bloomfield, NJ) for 7 d at 2°C. Packages were rotated twice daily in order to maintain a random sample placement.

Color Measurements

Trained visual color panelists (n=10) evaluated initial color on d 0 of display and display color and surface discoloration on d 0 to 7 of display once each day. Initial color was

determined using the following scale: 1) purplish red or reddish tan of vacuum package, 2) bleached, pale red, 3) slightly cherry red, 4) moderately light cherry red, 5) cherry red, 6) slightly dark red, 7) moderately dark red, 8) dark red, 9) very dark red. The color scale used by panelists for steaks packaged in MAP was: 1) very bright red or very bright pinkish red, 2) bright red or bright pinkish red, 3) dull red or dull pinkish red, 4) slightly dark red or slightly dark pinkish red, 5) reddish tan or pinkish tan, 6) moderately dark red or reddish tan or moderately dark pinkish red or pinkish tan, 7) tannish red or tannish pink, 8) tan to brown. Steaks packaged in VP were evaluated with the following scale: 1) very bright purplish red or very bright purplish pink, 2) bright purplish red or bright purplish pink, 3) dull purplish red or dull purplish pink, 4) slightly dark purplish red or slightly dark purplish pink, 5) purplish tan or pinkish tan, 6) moderately dark purplish red or moderately dark purplish pink, 7) tannish purple red or tannish purple pink, and 8) tan to brown. For all steaks, discoloration was considered as a percentage of surface metmyoglobin and the following scale was used to evaluate this: 1) none (0%), 2) slight discoloration (1-19%), 3) small discoloration (20-39%), 4) modest discoloration (40-59%), 5) moderate discoloration (60-79%), 6) extensive discoloration (80-99%), and 7) total discoloration (100%). Color scales were used to half-point increments and discoloration was scored to whole-point increments.

Statistical Analysis

The experimental design was a split plot design, with block being enhancement for animal. The subplot consisted of steaks from each muscle. The MIXED procedure of SAS was used to analyze the data. The fixed effects for WBSF were the comparison of mean of d 7 to mean of all other data, muscle, enhancement(the comparison of mean of d 7 to mean of all other data), enhancement ×muscle(the comparison of mean of d 7 to mean of all other data), packaging treatment(the comparison of mean of d 7 to mean of all other data), day(the comparison of mean of d 7 to mean of all other data), packaging treatment × day(the comparison of mean of d 7 to mean of all other data), day × muscle(the comparison of mean of d 7 to mean of all other data), day × enhancement(the comparison of mean of d 7 to mean of all other data), packaging treatment × muscle(the comparison of mean of d 7 to mean of all other data), packaging treatment × enhancement(the comparison of mean of d 7 to mean of all other data), packaging treatment × muscle × enhancement(the comparison of mean of d 7 to mean of all other data), day

× muscle × enhancement(the comparison of mean of d 7 to mean of all other data), day × packaging treatment × enhancement(the comparison of mean of d 7 to mean of all other data), day × packaging treatment × muscle(the comparison of mean of d 7 to mean of all other data), and day × packaging treatment × muscle × enhancement(the comparison of mean of d 7 to mean of all other data). The fixed effects for cook loss were enhancement, muscle, enhancement × muscle, packaging treatment, packaging treatment × muscle, packaging treatment × enhancement, and packaging treatment × muscle × enhancement. The fixed effects for desmin included day, enhancement × muscle(day), packaging treatment(day), and packaging treatment × muscle × enhancement(day). The fixed effects for pH and initial color were enhancement, muscle, enhancement × muscle, packaging treatment, packaging treatment × muscle, packaging treatment × enhancement, and packaging treatment × muscle × enhancement. The fixed effects for visual color and discoloration included enhancement, muscle, enhancement × muscle, packaging treatment, day, packaging treatment × day, day × muscle, day × enhancement, packaging treatment × muscle, packaging treatment × enhancement, packaging treatment × muscle × enhancement, day × packaging treatment × muscle, and day × packaging treatment × muscle × enhancement. Random effects for WBSF, cook loss, sensory traits, desmin degradation, pH, initial color, display color, and discoloration included animal, enhancement(animal), and muscle × animal(enhancement). Means were separated using Fisher's Protected LSD with Prasad-Rao-Jeske-Kackar-Harville standard errors and the Kenward-Roger degrees of freedom. Highest order interactions were reported when they were significant or main effects when no interactions were significant. Significance was determined at probability values of $P < 0.05$.

RESULTS AND DISCUSSION

Injection-enhancement targeted pump levels were 10% and after approximately 30 min of initial injection and just prior to fabrication, pump level was 10.7% for the longissimus lumborum, 8.2% for the semitendinosus, and 13.0% for the triceps brachii. Differences may have occurred due to inherent water holding capacity, muscle fiber type, collagen content, and/or muscle fiber orientation relative to the injection needles.

pH

There was a muscle × enhancement treatment interaction ($P < 0.05$) for pH (Table 3.1). Enhanced steaks had a higher ($P < 0.05$) pH than non-enhanced steaks regardless of packaging treatment. The lactate and/or phosphate in the enhancement solution caused an increase in muscle pH. The increased pH in enhanced steaks may be responsible for decreased cook loss and increased juiciness found in enhanced steaks compared to non-enhanced steaks. There is increased water-holding capacity of meat the further the pH is from the isoelectric point (5.1) of meat.

Table 3.1 pH muscle × enhancement treatment means and SE[a] for steaks

Muscle	Non-enhanced	Enhanced
Longissimus lumborum	5.5[b]	5.8[c]
Semitendinosus	5.6[b]	5.8[c]
Triceps brachii	5.6[b]	5.8[c]

[a]SE=0.02
[bc]Means with different superscript letters differ ($P < 0.05$)

Warner-Bratzler shear force

Tenderness, according to WBSF, for non-enhanced steaks resulted in similar results as in Chapter 2 in terms of the effect of packaging treatment and d postmortem in which there was a packaging treatment × d interaction ($P = 0.002$) (Table 3.2; Appendix B – Figure B.1). As found in Chapter 2, steaks packaged in HiO_2 MAP were less tender at the end of their display (d 18 postmortem) than steaks packaged in VP or ULO_2CO MAP at the end of their display (d 28 postmortem). Because there was no difference on d 14 postmortem, I attribute this difference in tenderness primarily to the fewer days postmortem associated with HiO_2 MAP at the end of display than VP or ULO_2CO MAP. This is due to storage differences commonly found in industry and described previously.

The results of other studies show more distinct differences in tenderness due to packaging environment. Steaks packaged in HiO_2 MAP have been shown to be less tender after 7 to 14 d than steaks packaged in VP or ULO_2, with or without CO MAP, by instrumental and/or trained sensory panelists (Tørngren, 2003; Sørheim et al., 2004; Clausen, 2004; and Madsen and Clausen, 2006). These studies used steaks from heifers, cows, or bulls and were most likely fed

different types of diets than the traditional grain-fed diets most often found in U.S. cattle harvested, such as the A-maturity, USDA Select carcasses used in my study. The differences in cattle gender, age, and feeding regimens between my study and others may have played a role in the results I saw compared to the other studies. Clausen (2004) believes that the detrimental effects of O_2 on tenderness may be caused by protein oxidation. Rowe et al. (2004) found that oxidation of beef muscle proteins early postmortem inactivated μ-calpain and decreased myofibrillar proteolysis, thus potentially limiting tenderization.

Table 3.2 Warner-Bratzler shear force packaging treatment × d means and SE for steaks packaged in different atmospheres

Packaging	d postmortem		
Treatment[a]	7	14	18/28
HiO_2	4.79[g]	4.01[f]	3.79[e]
ULO_2CO	4.79[g]	3.98[f]	3.39[d]
VP	4.79[g]	3.93[ef]	3.30[d]

[a]HiO_2 (80% O_2, 20% CO_2); ULO_2CO (0.4% CO/35% CO_2/64.6%N_2); VP (vacuum packaging)
[b]SE=0.08
[c]d 18 postmortem for the HiO_2 treatment and d 28 postmortem for the ULO_2CO and VP treatments
[defg]Means with different superscript letters differ ($P < 0.05$)

Although there was a trend ($P = 0.057$) for a muscle × enhancement × packaging treatment × d interaction for WBSF, there was a muscle × enhancement treatment × d significant interaction ($P = 0.03$) in which steaks from enhanced muscles were more tender ($P < 0.05$) than non-enhanced steaks (Table 3.3; Appendix B – Figure B.2). Tenderness increased with time postmortem (d 14 to 18/28) in enhanced longissimus lumborum and triceps brachii steaks but not in semitendinosus steaks. Non-enhanced steaks were similar in tenderness on d 7 and 14 postmortem and were more tender on d 18/28 postmortem for all muscles. Enhanced longissimus lumborum steaks were more tender ($P < 0.05$) than non-enhanced steaks on d 7 postmortem, which was d 0 of packaging. This indicates that injection-enhancement has an immediate effect on tenderness. Injection-enhancement may increase tenderness through a dilution effect or through physically altering the muscle structure with the injection needling process; however, the exact method of action is currently unknown. Due to time constraints and

cooking capabilities, I was unable to determine WBSF on d 7 postmortem (d of fabrication and d 0 of packaging) for enhanced semitendinosus and triceps brachii muscles.

Other studies have found that injection-enhancement of beef muscles results in decreased shear force values compared to non-enhanced steaks. Wicklund et al. (2005) reported that enhanced beef strip loin steaks had lower WBSF values than non-enhanced steaks when loins were aged before enhancement and when loins were enhanced before aging. Vote et al. (2000) and Robbins et al. (2003) reported lower shear force in enhanced beef strip loin steaks than in non-enhanced steaks. Lawrence et al. (2004) evaluated injection of beef longissimus muscles with a phosphate and salt solution or a calcium lactate plus beef broth or carrageenan with rosemary extract solution and found no differences in WBSF among all treatments. Knock et al. (2006a) reported that steaks enhanced with sodium acetate had lower shear force than control steaks or steaks enhanced with potassium lactate but the mechanism for this difference is unknown.

Table 3.3 Warner-Bratzler shear force muscle $\times$ enhancement treatment $\times$ d means and SE

Muscle	d postmortem	Non-enhanced	SE	Enhanced	SE
Longissimus lumborum	7	4.78[hi]	0.18	3.24[d]	0.18
Longissimus lumborum	14	4.59[h]	0.13	2.70[c]	0.13
Longissimus lumborum	18/28[a]	3.92[fg]	0.13	2.35[b]	0.13
Semitendinosus	7	5.03[i]	0.18	-	-
Semitendinosus	14	5.11[i]	0.13	3.66[ef]	0.13
Semitendinosus	18/28[a]	4.43[h]	0.13	3.41[de]	0.13
Triceps brachii	7	4.55[h]	0.13	-	-
Triceps brachii	14	4.45[h]	0.11	3.33[de]	0.11
Triceps brachii	18/28	4.08[g]	0.11	2.75[c]	0.11

[a]d 18 postmortem for the HiO$_2$ treatment and d 28 postmortem for the ULO$_2$CO and VP treatments
[bcdefghi]Means with different superscript letters differ ($P < 0.05$)

Sensory analysis

There was an enhancement treatment $\times$ packaging treatment interaction for myofibrillar tenderness ($P < 0.03$), beef flavor and off-flavor ($P < 0.001$), and overall tenderness ($P < 0.02$) (Table 3.4; Appendix B – Figures B.3 and B.4). According to sensory panelists, non-enhanced steaks packaged in HiO$_2$ MAP were less tender, had less beef flavor, and more off-flavors ($P <$

0.05) than those packaged in ULO_2CO MAP and VP. The longissimus lumborum (5.9 ± 0.1) and triceps brachii (6.0 ± 0.1) were more tender according to myofibrillar tenderness ($P < 0.05$) than the semitendinosus (5.1 ± 0.1). Enhanced steaks packaged in VP had more ($P < 0.05$) beef flavor than enhanced steaks packaged in HiO_2 MAP.

The main effect ($P = 0.001$) for juiciness revealed that enhanced steaks (5.7 ± 0.1) were juicier ($P < 0.05$) than non-enhanced steaks (5.1 ± 0.1). These results agree with cooking loss data and were expected because enhanced steaks have additional moisture added to them at the time of injection. The muscle main effect ($P < 0.001$) for juiciness resulted in steaks from longissimus lumborum (5.5 ± 0.2) and triceps brachii (5.9 ± 0.1) muscles being juicier ($P < 0.05$) than steaks from semitendinosus (5.0 ± 0.2) muscles. There was a packaging treatment main effect ($P < 0.01$) for juiciness. Steaks packaged in HiO_2 MAP (5.3 ± 0.1) were less juicy ($P < 0.05$) than steaks packaged in ULO_2CO MAP (5.6 ± 0.1), whereas steaks packaged in VP (5.4 ± 0.1) were intermediate and not different in juiciness from steaks packaged in HiO_2 and ULO_2CO MAP.

There was a main effect ($P < 0.001$) for connective tissue for the enhancement treatment and muscle. Enhanced (6.6 ± 0.1) steaks had less ($P < 0.05$) perceptible connective tissue than non-enhanced (6.1 ± 0.1) steaks. The semitendinosus (5.9 ± 0.1) had more ($P < 0.05$) perceptible connective tissue than the triceps brachii (6.4 ± 0.1), which had more ($P < 0.05$) perceptible connective tissue than the longissimus lumborum (6.7 ± 0.1). There was also a main effect ($P < 0.001$) for packaging treatment for connective tissue in which steaks packaged in HiO_2 MAP (6.2 ± 0.1) had more ($P < 0.05$) perceptible connective tissue than steaks packaged in ULO_2CO MAP (6.4 ± 0.1) and VP (6.4 ± 0.1).

Table 3.4 Sensory enhancement × packaging treatment means and SE[a] for steaks packaged in different atmospheres

Packaging treatment[e]	Myofibrillar tenderness[b] Non-enhanced	Enhanced	Beef flavor[c] Non-enhanced	Enhanced	Overall tenderness[b] Non-enhanced	Enhanced	Off-flavor[d] Non-enhanced	Enhanced
HiO$_2$	4.6^f	6.2^h	4.3^{fg}	4.1^f	4.8^f	6.2^h	5.4^g	5.1^f
ULO$_2$CO	5.1^g	6.4^h	5.1^h	4.3^{fg}	5.3^g	6.4^h	7.0^h	5.3^g
VP	5.1^g	6.3^h	5.2^h	4.4^g	5.3^g	6.3^h	7.0^h	5.5^g

[a]SE=0.1 for myofibrillar and overall tenderness and off-flavor; SE=0.07 for beef flavor
[b]1=extremely tough, 4=slightly tough, 6=moderately tender, 8=extremely tender
[c]1=extremely bland, 4=slightly bland, 6=moderately intense, 8=abundant
[d]1=abundant, 5=slight, 6=traces, 7=practically none, 8=none
[e]HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (0.4% CO/35% CO$_2$/64.6%N$_2$); VP (vacuum packaging)
[fgh]Means with different superscript letters within sensory traits differ ($P < 0.05$)

Table 3.5 Sensory muscle × enhancement treatment means and SE[a] for beef flavor and off-flavor of steaks

Muscle	Beef Flavor[b] Non-enhanced	Enhanced	Off-flavor[c] Non-Enhanced	Enhanced
Longissimus lumborum	5.0^g	4.3^{de}	6.4^f	5.1^d
Semitendinosus	4.6^f	4.1^d	6.4^f	5.6^e
Triceps brachii	5.1^g	4.4^e	6.4^f	5.2^d

[a]SE=0.07 for beef flavor and SE=0.1 for off-flavor
[b]1=extremely bland, 4=slightly bland, 6=moderately intense, 8=abundant
[c]1=abundant, 5=slight, 6=traces, 7=practically none, 8=none
[defgh]Means with different superscript letters within sensory traits differ ($P < 0.05$)

The most common off-flavors associated with steaks packaged in HiO_2 MAP were oxidative or rancid. Enhanced steaks all had more ($P < 0.05$) off-flavors than non-enhanced steaks, with typical descriptors of salty and metallic or chemical. There were also comments on many of the enhanced steaks that indicated an undesirable mushy texture. There was a muscle $\times$ enhancement treatment interaction for beef flavor ($P = 0.038$) and off-flavor ($P = 0.046$) (Table 3.5; Appendix A – Figure B.5). Enhanced triceps brachii steaks had more ($P < 0.05$) beef flavor than enhanced semitendinosus steaks. Oxidative off-flavors associate with steaks packaged in HiO_2 MAP were expected because the O_2 present in the package atmosphere allows for more rapid and a greater extent of oxidation of proteins and lipids found in meat. Eliminating O_2 from the package environment, as done with VP or ULO_2CO MAP, drastically decreases the rate and extent of oxidation, thus resulting in fewer off-flavors and increased beef flavor.

Our results for beef-flavor intensity are similar to those of Carmack et al. (1995) who reported that the triceps brachii were equal in beef-flavor intensity to the longissimus lumborum and had more flavor than the semitendinosus. They found the longissimus lumborum to have as much beef-flavor intensity as the semitendinosus, but in my study, steaks from the semitendinosus had less beef-flavor than steaks from the triceps brachii and the longissimus lumborum. Carmack et al. (1995) reported that the longissimus lumborum was more tender than the triceps brachii, which was equal in tenderness to the semitendinosus. In my study, the triceps brachii was equal in tenderness to the longissimus lumborum and both were more tender than the semitendinosus according to trained sensory panelists. Carmack et al. (1995) reported that the longissimus lumborum was juicier than the semitendinosus, with the triceps brachii being similar in juiciness to both. My results indicate that the triceps brachii and longissimus lumborum were juicier than the semitendinosus.

Jackson et al. (1992) evaluated the volatile compounds from the headspace of beef strip loins vacuum packaged or in: 100% CO_2 MAP; ULO_2 MAP; or HiO_2 MAP. They reported that steaks packaged in HiO_2 MAP developed strong off-odors and had methyl thiirane, ethyl acetate, benzene and 1-heptene in the packages after 7 and 14 d of storage but not in the vacuum packaged steaks or other MAP atmospheres.

Seyfert et al. (2005) reported that beef quadriceps injection-enhanced and packaged in HiO_2 MAP were less tender and had more off-flavors than those in ULO_2 MAP. They also reported that increasing injection percentage from 6 to 10% in beef round muscles decreased

oxidation but increased non-typical beef flavors. Hoffman (2006) found that enhancement of cow longissimus and semitendinosus muscles resulted in more tender and juicier steaks that were saltier and had less overall beef flavor than steaks that were non-enhanced. Wicklund et al. (2005) found similar results in beef loin steaks.

Knock et al. (2006a) found that adding potassium lactate to injection-enhanced beef packaged in HiO_2 MAP limited rancid flavor development while increasing brown-roasted and beef flavors. They also found that increasing the salt content in the injection-enhancement solution increased salty and rancid flavors. In addition, oxidized, stale and rancid flavors increased as time in HiO_2 MAP increased. Vote et al. (2000) reported increased tenderness and juiciness for injected steaks compared to control non-injected steaks. They also found a trend for increased cooked beef flavor, but when sodium tripolyphosphate was injected alone, soapy and sour off-flavors were detected. They also cooked steaks to either 66 or 77°C and showed that the improvements in tenderness and juiciness compared to control steaks were even greater at 77°C. Thus, injection enhancement may be beneficial when consumers overcook steaks, and it helps processors deliver more consistent products to their consumers.

Lawrence et al. (2003a) evaluated injection marination of beef longissimus muscles with calcium ascorbate, calcium chloride, or calcium lactate and reported that calcium lactate increased beef flavor and had no effect on off flavor compared to control samples. Lawrence et al. (2003b) injected beef longissimus and semitendinosus muscles with calcium lactate followed by a phosphate and salt injection solution. They found no differences in tenderness or sensory traits for the semitendinosus muscles but found increased tenderness for injected longissimus muscles compared to control muscles. They reported that longissimus steaks from muscles injected with phosphate and salt in addition to calcium lactate had less beef flavor and fewer complaints of samples being too salty than steaks from control muscles or muscles only injected with calcium lactate. Trained sensory panelists found that steaks enhanced with calcium lactate plus rosemary were less tender than steaks enhanced with phosphate and salt plus rosemary, but off flavors of metallic and salty were increased with the phosphate and salt plus rosemary treatment compared to the calcium lactate plus rosemary treatment (Lawrence et al., 2004).

Stetzer et al. (2007) compared beef loin steaks injection-enhanced with phosphate, salt, and natural flavorings and packaged in HiO_2 MAP and CO MAP and reported that consumer

sensory panelists did not find differences due to packaging environment in beef flavor, off flavor, or overall acceptability.

Cooking loss

There were no interactions for cooking loss, but there were main effects ($P < 0.001$) for muscle, enhancement treatment, and packaging treatment ($P = 0.006$). The longissimus lumborum (19.7% $\pm$ 0.5) had the least ($P < 0.05$) cooking loss, the triceps brachii (22.7% $\pm$ 0.5) had intermediate cooking loss, and the semitendinosus had the most ($P < 0.05$) cooking loss (26.9% $\pm$ 0.5). Enhanced steaks (19.9% $\pm$ 0.4) had less ($P < 0.05$) cooking loss than non-enhanced steaks (26.3% $\pm$ 0.4). Steaks packaged in HiO$_2$ MAP (22.1% $\pm$ 0.5) had the least ($P < 0.05$) cooking loss and steaks packaged in ULO$_2$CO MAP (24.1% $\pm$ 0.5) had the most ($P < 0.05$) cooking loss. Cooking loss of vacuum packaged steaks (23.2% $\pm$ 0.5) was intermediate and not different ($P > 0.05$) than cooking loss of steaks packaged in HiO$_2$ or ULO$_2$CO MAP.

Wicklund et al. (2005) reported that enhanced beef strip loin steaks had less cooking loss on d 7 of storage but not on d 14, 21, or 28 than non-enhanced steaks. Molina et al. (2005) found that enhancement by marination, needle-pumping, and vacuum-tumbling resulted in decreased cooking loss compared to non-enhanced steaks from several different muscles from the beef chuck. Stetzer et al. (2007) compared beef loin steaks injection-enhanced with phosphate, salt, and natural flavorings and packaged in HiO$_2$ MAP and CO MAP and reported no differences in purge loss or cooking loss when both packaging methods were stored for 14 d, but steaks in CO MAP had more cooking loss when stored for 28 d compared to steaks in both packaging types at 14 d of storage.

Desmin degradation

There was a muscle $\times$ enhancement(d) interaction ($P < 0.001$) for desmin degradation (Table 3.5; Appendix B – Figure B.6). Non-enhanced and enhanced steaks were not different ($P > 0.05$) in the amount of desmin degradation. Longissimus lumborum desmin degradation increased ($P < 0.05$) from d 7 to d 14, regardless of enhancement treatment. Longissimus lumborum steaks had more ($P < 0.05$) degradation of desmin at d 14 than the semitendinosus or triceps brachii, regardless of enhancement treatment. Different muscles varying in rates and extent of postmortem proteolysis, of which desmin degradation is a key determinant of tenderness (Koohmaraie and Geesink, 2006). Desmin degradation was not affected ($P > 0.05$)

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by type of packaging but was affected ($P < 0.05$) by time postmortem. There was a d postmortem main effect ($P < 0.001$) for desmin degradation, with d 14 postmortem (36.09% ± 2.9) having more ($P < 0.05$) degradation than d 7 (23.67% ± 3.3).

Table 3.6 Desmin degradation (%) muscle × enhancement(d) means and SE for steaks packaged in different atmospheres

Muscle	Day	Non-enhanced	Enhanced	SE
Longissimus lumborum	7	28.18[bc]	34.20[c]	4.86
Longissimus lumborum	14	47.21[d]	54.22[d]	4.30
Semitendinosus	7	19.25[ab]	-	4.85
Semitendinosus	14	31.43[c]	33.91[c]	4.29
Triceps brachii	7	13.03[a]	-	3.92
Triceps brachii	14	21.74[b]	28.06[bc]	3.67

[abcd]Means with different superscript letters differ ($P < 0.05$)

      Increased desmin degradation with increased d postmortem was expected because aging increases postmortem proteolysis and the breakdown of desmin. Furthermore, I did not expect to find differences in desmin degradation for enhancement treatments and my results agree with this. I hypothesized that packaging treatment may alter desmin degradation through protein oxidation associated with HiO_2 MAP, by slowing down or hindering postmortem proteolysis as indicated by Rowe et al. (2004); my results did not indicate this. Protein oxidation may be associated with muscle in the early stages after harvest. Muscles used in my study were aged for 7 d in vacuum prior to exposing steaks to different packaging treatments. To my knowledge, my study is the first to look at desmin degradation for enhanced and non-enhanced steaks from different muscles in different packaging atmospheres. An example of a Western blot of desmin degradation is shown in Figure 3.1.

Figure 3.1 An example of a Western blot for longissimus lumborum samples from an animal that had non-enhanced (N) and enhanced (E) steaks packaged in HiO_2 (80% O_2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); VP (vacuum packaging) or control (d7) with a standard (std)
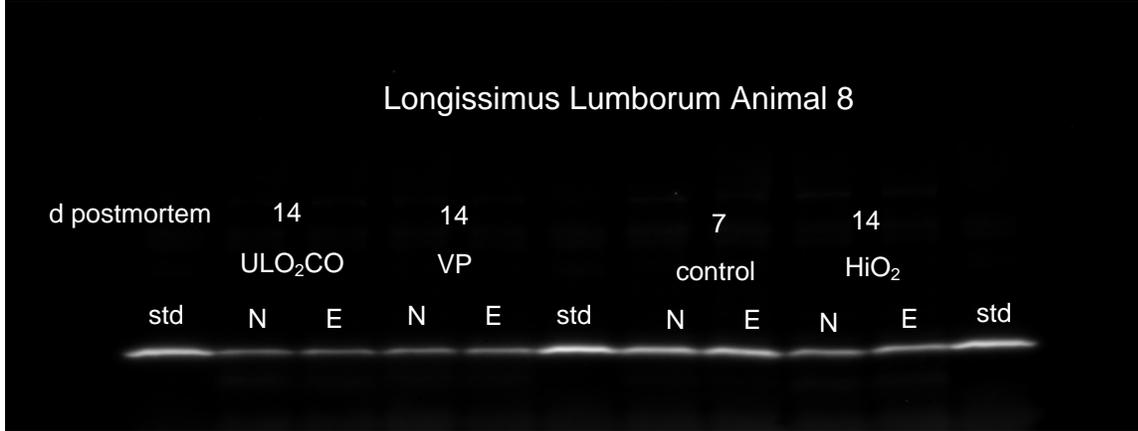
Display color and discoloration

There was a muscle × packaging treatment interaction ($P < 0.001$) for initial color score (Table 3.7). The initial color for semitendinosus steaks was lighter cherry red ($P < 0.05$) than for longissimus lumborum or triceps brachii steaks. Triceps brachii steaks packaged in HiO_2 MAP were darker red ($P < 0.05$) than triceps brachii steaks packaged in ULO_2CO MAP initially.

Table 3.7 Initial color score[a] muscle × packaging treatment means and SE[b] for steaks

Muscle	HiO_2[c]	ULO_2CO[c]	VP[c]
Longissimus lumborum	5.7[g]	5.8[g]	2.0[d]
Semitendinosus	4.8[f]	4.9[f]	2.2[d]
Triceps brachii	6.6[h]	6.1[g]	3.2[e]

[a]1=purplish red or reddish tan of vacuum package, 4=moderately light cherry red, 5=cherry red, 6=slightly dark red
[b]SE=0.19
[c]HiO_2 (80% O2, 20% CO_2); ULO_2CO (0.4% CO/35% CO_2/64.6%N_2); VP (vacuum packaging)
[defgh]Means with different superscript letters differ ($P < 0.05$)

Enhanced steaks were darker ($P < 0.05$) initially than non-enhanced steaks. Lactate in the enhancement solution is typically associated with increased color stability but also results in slightly darker muscle color (Kim et al., 2006). Non-enhanced triceps brachii steaks were darker

($P < 0.05$) than non-enhanced longissimus lumborum or semitendinosus steaks. Enhanced semitendinosus steaks were lighter ($P < 0.05$) than enhanced longissimus lumborum steaks, which were lighter ($P < 0.05$) than enhanced triceps brachii steaks. Differences in muscle fiber type most likely caused differences in initial color among muscles and within packaging treatments.

There was a muscle × enhancement treatment × packaging treatment × d interaction ($P < 0.02$) for display color scores (Table 3.8; Appendix B – Figure B.7). Steaks became darker ($P < 0.05$) throughout the 7 d of display, but in general, steaks packaged in VP or ULO_2CO MAP remained more stable than steaks packaged in HiO_2 MAP. These results agree with results from Chapter 2. In general, triceps brachii steaks were darker than longissimus lumborum and semitendinosus steaks. Non-enhanced triceps brachii steaks packaged in HiO_2 MAP became dramatically darker than those packaged in ULO_2CO MAP and VP. Steaks packaged in HiO_2 or ULO_2CO MAP tended to become darker in color at a faster rate than steaks in VP.

Table 3.8 Display color score muscle × enhancement treatment × packaging treatment × d means and SE[a] for non-enhanced or enhanced longissimus lumborum, semitendinosus, or triceps brachii steaks packaged in different atmospheres and displayed

	Visual color scores							
	d of display							
	0	1	2	3	4	5	6	7
Longissimus lumborum								
Non-enhanced								
HiO$_2$[bc]	2.4^{e,y}	2.8^{f,y}	3.3^{g,y}	3.3^{g,z}	3.5^{g,y}	4.0^{h,y}	4.5^{i,y}	5.0^{j,x}
ULO$_2$CO[bc]	3.2^{e,z}	3.2^{e,y}	3.5^{ef,y}	3.7^{f,z}	4.0^{g,z}	3.7^{f,y}	4.2^{g,y}	4.4^{h,y}
VP[bd]	2.9^{fg,z}	2.5^{e,z}	2.8^{f,z}	3.3^{h,z}	3.6^{i,yz}	3.2^{gh,z}	3.7^{i,z}	3.9^{i,z}
Enhanced								
HiO$_2$[bc]	4.2^{e,x}	4.2^{e,x}	4.6^{f,x}	4.8^{f,x}	4.7^{f,x}	4.7^{f,x}	5.3^{g,y}	5.5^{g,x}
ULO$_2$CO[bc]	5.0^{e,y}	4.8^{e,y}	5.1^{e,y}	5.2^{ef,y}	5.2^{ef,y}	5.9^{g,y}	5.5^{fg,y}	5.6^{g,y}
VP[bd]	3.4^{e,z}	3.5^{e,z}	3.7^{ef,z}	4.1^{gh,z}	4.2^{h,z}	3.9^{fg,z}	4.6^{i,z}	4.9^{j,z}
Semitendinosus								
Non-enhanced								
HiO$_2$[bc]	2.5^{e,y}	2.9^{f,z}	3.1^{f,z}	3.7^{g,y}	4.3^{h,y}	5.2^{i,y}	5.3^{i,x}	5.5^{i,x}
ULO$_2$CO[bc]	3.1^{e,z}	3.0^{e,z}	3.0^{ef,z}	3.4^{fg,y}	3.7^{gh,z}	3.6^{g,z}	4.0^{hi,y}	4.2^{i,y}
VP[bd]	2.8^{ef,yz}	2.6^{e,z}	2.8^{ef,z}	3.1^{f,yz}	3.6^{g,z}	3.4^{g,z}	3.5^{g,z}	3.5^{g,z}
Enhanced								
HiO$_2$[bc]	3.2^{e,y}	3.4^{e,y}	3.8^{f,y}	4.6^{g,y}	4.6^{g,y}	5.0^{h,x}	5.5^{i,x}	5.6^{i,x}
ULO$_2$CO[bc]	3.8^{e,z}	4.0^{f,z}	4.2^{f,y}	4.4^{fg,y}	4.8^{hi,y}	4.6^{gh,y}	4.9^{i,y}	5.0^{i,y}
VP[bd]	3.2^{e,y}	3.2^{e,y}	3.3^{e,z}	3.8^{f,z}	4.1^{gh,z}	3.9^{fg,z}	4.2^{h,z}	4.3^{h,z}
Triceps brachii								
Non-enhanced								
HiO$_2$[bc]	3.6^{e,y}	3.9^{e,y}	4.3^{f,x}	5.2^{g,x}	5.8^{h,x}	6.5^{i,x}	6.6^{i,y}	7.0^{j,x}
ULO$_2$CO[bc]	3.8^{e,y}	3.6^{e,y}	3.8^{e,y}	4.2^{f,y}	4.4^{fg,y}	4.2^{f,y}	4.5^{fg,z}	4.7^{g,y}
VP[bd]	3.3^{ef,z}	3.0^{e,z}	3.3^{ef,z}	3.6^{gh,z}	3.8^{hi,z}	3.4^{fg,z}	4.1^{i,z}	4.0^{hi,z}
Enhanced								
HiO$_2$[bc]	4.8^{e,y}	5.3^{fg,y}	5.1^{ef,yz}	5.6^{g,y}	6.0^{h,y}	6.2^{hi,y}	6.2^{hi,y}	6.4^{i,y}
ULO$_2$CO[bc]	5.3^{e,z}	5.5^{e,y}	5.5^{e,y}	5.6^{ef,y}	5.8^{f,y}	5.9^{f,y}	5.9^{f,yz}	5.9^{f,z}
VP[bd]	4.7^{e,y}	4.7^{ef,z}	4.8^{ef,z}	5.0^{f,z}	5.1^{f,z}	4.9^{ef,z}	5.5^{g,z}	5.6^{g,z}

[a]SE=0.18

[b]HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); VP (vacuum packaging)

[c]2=bright red or bright pinkish red, 3=dull red or dull pinkish red, 4=slightly dark red or slightly dark pinkish red, 5=reddish tan or pinkish tan, 6=moderately dark red or reddish tan or moderately dark pinkish red or pinkish tan, 7= tannish red or tannish pink

[d]2=bright purplish red or bright purplish pink, 3=dull purplish red or dull purplish pink, 4=slightly dark purplish red or slightly dark purplish pink, 5=purplish tan or pinkish tan,

[efghij]Means within the same treatment row with different superscript letters differ ($P < 0.05$)

[xyz]Means within columns within muscle and enhancement treatment with different superscript letters differ ($P < 0.05$)

There was a muscle $\times$ enhancement treatment $\times$ packaging treatment $\times$ d interaction ($P <$ 0.001) for discoloration scores (Table 3.9; Appendix B – Figure B.8). Steaks packaged in HiO_2 MAP discolored at a relatively fast rate ($P < 0.05$) and to a greater extent ($P < 0.05$) than steaks packaged in VP or ULO_2CO MAP. Steaks packaged in VP or ULO_2CO MAP had no ($P < 0.05$) discoloration throughout the 7 d display. Including O_2 in the package allows for oxidation of myoglobin (main pigment in meat that gives it color) and thus resulting in a reddish tan color by d 7 of display. Excluding O_2 from the package, as with VP or ULO_2CO MAP treatments, allows myoglobin to remain in a more stable form longer and delays the onset of metmyoglobin (tan/brown) color formed through the oxidation of myoglobin. In general, triceps brachii steaks packaged in HiO_2 MAP discolored at a faster rate and to a greater extent than longissimus lumborum and semitendinosus steaks packaged in HiO_2 MAP, regardless of enhancement treatment.

Table 3.9 Discoloration score muscle × enhancement treatment × packaging treatment × d means and SE[a] for non-enhanced or enhanced longissimus lumborum, semitendinosus, or triceps brachii steaks packaged in different atmospheres and displayed

	Discoloration score[b]							
	d of display							
	0	1	2	3	4	5	6	7
Longissimus lumborum								
Non-enhanced								
HiO_2[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.3^{e,z}$	$1.5^{f,y}$	$2.0^{g,y}$	$2.5^{h,y}$
ULO_2CO[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$
VP[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$
Enhanced								
HiO_2[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.4^{e,y}$	$1.7^{f,y}$
ULO_2CO[c]	$1.0^{d,z}$	$1.1^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,yz}$	$1.1^{d,z}$
VP[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$
Semitendinosus								
Non-enhanced								
HiO_2[c]	$1.2^{d,z}$	$1.3^{d,z}$	$1.5^{e,y}$	$1.5^{e,y}$	$2.1^{f,y}$	$2.2^{g,y}$	$2.7^{h,y}$	$3.0^{i,y}$
ULO_2CO[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$
VP[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$
Enhanced								
HiO_2[c]	$1.1^{d,z}$	$1.3^{e,z}$	$1.7^{f,y}$	$1.7^{f,y}$	$1.9^{g,y}$	$2.0^{g,y}$	$2.5^{h,y}$	$3.0^{i,y}$
ULO_2CO[c]	$1.0^{d,z}$	$1.1^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.2^{d,z}$	$1.1^{d,z}$	$1.2^{d,z}$
VP[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$
Triceps brachii								
Non-enhanced								
HiO_2[c]	$1.1^{d,z}$	$1.3^{e,z}$	$1.6^{f,y}$	$1.6^{f,y}$	$3.2^{g,y}$	$4.1^{h,y}$	$4.6^{i,y}$	$5.2^{j,y}$
ULO_2CO[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$
VP[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$
Enhanced								
HiO_2[c]	$1.2^{e,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.1^{de,z}$	$1.7^{f,y}$	$2.3^{g,y}$	$3.2^{h,y}$	$3.6^{i,y}$
ULO_2CO[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.1^{d,z}$	$1.1^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$
VP[c]	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$	$1.0^{d,z}$

[a]SE=0.12

[b]1=none (0%), 2=slight discoloration (1-19%), 3=small discoloration (20-39%), 4=modest discoloration (40-59%), 5=moderate discoloration (60-79%), 6=extensive discoloration (80-99%), 7=total discoloration (100%)

[c]HiO_2 (80% O_2, 20% CO_2); ULO_2CO (64.6% N_2, 35% CO_2, 0.4% CO); and VP (vacuum packaging)

[defghij]Means within the same treatment row with different superscript letters differ ($P < 0.05$)

[yz]Means within the same column within muscle and enhancement treatment with different superscript letters differ ($P < 0.05$)

Knock et al. (2006b) injected beef longissimus thoracis muscles with different combinations of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate and packaged as steaks in HiO_2 MAP. They found that steaks from muscles injected with potassium lactate, with or without sodium acetate, had increased color stability but were darker than control steaks. Lawrence et al. (2004) evaluated injection of beef longissimus muscles and reported improvements in display color stability when rosemary extract was included in the enhancement solution with calcium lactate than muscles injected with phosphate and salt with rosemary.

There have been other reports of beef stored in ULO_2CO MAP maintaining red color while steaks packaged in HiO_2 MAP discolor more rapidly. Behrends et al. (2003) reported acceptable color stability of steaks packaged in HiO_2 MAP through d 5 of display. Other researchers showed increased times of storage in HiO_2 MAP for steaks being red in color; however, some of these steaks were stored in dark storage and not displayed under lights as in my study. The use of 0.4% CO in retail meat packages was approved by USFDA (2004). John et al. (2005) reported that steaks in HiO_2 MAP were red in color through 14 d of storage and steaks in ULO_2CO MAP were red through 21 d of storage. They also found that the majority of steaks stored in VP remained purple in color through d 21 of storage; however, some of their VP steaks must have had residual oxygen in the package and turned brown in color. In addition, Sørheim et al. (1999) and Hunt et al. (2004) reported bright red color and high a* values of steaks stored in ULO_2CO MAP.

In summary, more off-flavors were associated with enhanced steaks than non-enhanced steaks. Enhanced steaks were darker in color, had a higher pH, were juicier, and had less perceptible connective tissue than non-enhanced steaks. Steaks packaged in HiO_2 MAP were less tender according to sensory panelists and had more off-flavors than either ULO_2CO MAP or VP. It is not clear why sensory panelists found steaks packaged in HiO_2 MAP to be less tender than steaks packaged in VP or ULO_2CO MAP on d 18 postmortem and WBSF results from steaks on d 14 postmortem were not different. Packaging treatment did not affect desmin degradation, which is a measure of tenderization during aging. In general, steaks packaged in VP or ULO_2CO MAP had more display color stability than steaks packaged in HiO_2 MAP. Regardless of enhancement treatment, steaks packaged in VP or ULO_2CO MAP did not discolor throughout 7 d of display, whereas steaks packaged in HiO_2 MAP discolored. Although steaks

packaged in VP did not discolor throughout display and had good color stability, they have a purplish red color that is not acceptable to most consumers. Packaging meat in ULO_2CO MAP is an effective way to package meat to maintain red color and minimize any negative effects on tenderness or sensory attributes.

LITERATURE CITED

AMSA. 1995. Research guidelines for cookery, sensory evaluation, and tenderness measurements of fresh meat. Am. Meat Sci. Assoc., Chicago, IL.

Behrends, J. M., W. B. Mikel, C. L. Armstrong, and M. C. Newman. 2003. Color stability of semitendinosus, semimembranosus, and biceps femoris steaks packaged in a high-oxygen modified atmosphere. J. Anim. Sci. 81:2230-2238.

Carmack, C. F., C. L. Kastner, M. E. Dikeman, J. R. Schwenke, and C. M. Garcia Zepeda. 1995. Sensory evaluation of beef-flavor-intensity, tenderness, and juiciness among major muscles. Meat Sci. 39:143-147.

Clausen, I. 2004. Sensory evaluation of beef loin steaks stored in different atmospheres. Proc. 50th Int. Congr. Meat Sci. Technol. Helsinki, Finland.

Hoffman, L. C. 2006. Sensory and physical characteristics of enhanced vs. non-enhanced meat from mature cows. Meat Sci. 72:195-202.

Hunt, M. C., R. A. Mancini, K. A. Hachmeister, D. H. Kropf, M. Merriman, G. DelDuca, and G. Milliken. 2004. Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorganisms of beef steaks and ground beef. J. Food Sci. 69(1):C45-C52.

Jackson, T. C., G. R. Acuff, C. Vanderzant, T. R. Sharp, and J. W. Savell. 1992. Identification and evaluation of volatile compounds of vacuum and modified atmosphere packaged beef strip loins. Meat Sci. 31:175-190.

John, L., D. Cornforth, C. E. Carpenter, O. Sorheim, B. C. Pettee, and D. R. Whittier. 2005. Color and thiobarbituric acid values of cooked top sirloin steaks packaged in modified atmospheres of 80% oxygen, or 0.4% carbon monoxide, or vacuum. Meat Sci. 69:441-449.

Kim, Y. H., M. C. Hunt, R. A. Mancini, M. Seyfert, T. M. Loughin, D. H. Kropf, and J. S. Smith. 2006. Mechanism for lactate-color stabilization in injection-enhanced beef. J. Ag. Food Chem. 54(20):7856-7862.

Knock, R. C., M. Seyfert, M. C. Hunt, M. E. Dikeman, R. A. Mancini, J. A. Unruh, J. J. Higgins, and R. A. Monderen. 2006a. Effects of potassium lactate, sodium chloride, and sodium acetate on surface shininess/gloss and sensory properties of injection-enhanced beef strip-loin steaks. Meat Sci. 74:319-326.

Knock, R. C., M. Seyfert, M. C. Hunt, M. E. Dikeman, R. A. Mancini, J. A. Unruh, J. J. Higgins, and R. A. Monderen. 2006b. Effects of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate on colour, colour stability, and oxidative properties of injection-enhanced beef rib steaks. Meat Sci. 74:312-318.

Koohmaraie, M. and G. H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci. 74(1):34-43.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003a. Effects of calcium salts on beef longissimus quality. Meat Sci. 64(2):299-308.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2004. Effects of enhancing beef *longissimus* with phosphate plus salt, or calcium lactate plus non-phosphate water binders plus rosemary extract. Meat Sci. 67:129-137.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003b. Staged injection marination with calcium lactate, phosphate and salt may improve beef water-binding ability and palatability traits. Meat Sci. 65:967-972.

Madsen, N. T., and I. Clausen. 2006. Sensory evaluation of beef steaks stored in MA combinations of CO, CO2, N2, and O2. Page 405-406 in Proc 52nd Int. Congr. Meat Sci. Technol., Dublin, Ireland.

Molina, M. E., D. D. Johnson, R. L. West, and B. L. Gwartney. 2005. Enhancing palatability traits in beef chuck muscles. Meat Sci. 71:52-61.

Robbins, K., J. Jensen, K. J. Ryan, C. Homco-Ryan, F. K. McKeith, and M. S. Brewer. 2003. Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks. Meat Sci. 64:279-285.

Rowe, L. J., K. R. Maddock, S. M. Lonergan, and E. Huff-Lonergan. 2004. Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. J. Anim. Sci. 82:3254-3266.

Seyfert, M., M. C. Hunt, R. A. Mancini, K. A. Hachmeister, D. H. Kropf, J. A. Unruh, and T. M. Loughin. 2005. Beef quadriceps hot boning and modified atmosphere packaging influence properties of injection-enhanced beef round muscles. J. Anim. Sci. 83:686-693.

Sørheim, O., H. Nissen, and T. Nesbakken. 1999. The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat Sci. 52:157-164.

Sørheim, O., M. Wahlgren, B. N. Nilsen, and P. Lea. 2004. Effects of high oxygen packaging on tenderness and quality characteristics of beef longissimus muscles. Proc. 50th Int. Congr. Meat Sci. Technol., Helsinki, Finland.

Stetzer, A. J., R. A. Wicklund, D. D. Paulson, E. M. Tucker, B. J. MacFarlane, and M. S. Brewer. 2007. Effect of carbon monoxide and high oxygen modified atmosphere packaging (MAP) on quality characteristics of beef strip steaks. J. Mus. Foods. 18:56-66.

Tørngren, M. A. 2003. Effect of packing method on colour and eating quality of beef loin steaks. Pages 495-496 in Proc. 49th Int. Congr. Meat Sci. Technol., Campinas, Brazil.

U. S. Food and Drug Administration (USFDA). 2004. GRAS notice number GRN 000143. Washington, D.C.:

Vote, D. J., W. J. Platter, J. D. Tatum, G. R. Schmidt, K. E. Belk, G. C. Smith, and N. C. Speer. 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. J. Anim. Sci. 78:952-957.

Wheeler, T. L., and M. Koohmaraie. 1999. The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. J. Anim. Sci. 77:2444-2451.

Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 2002. Technical note: sampling methodology for relating sarcomere length, collagen concentration, and the extent of postmortem proteolysis to beef and pork longissimus tenderness. J. Anim. Sci. 80:982-987.

Wicklund, S. E., C.Homco-Ryan, K. J. Ryan, F. K. McKeith, B. J. McFarlane, and M. S. Brewer. 2005. Aging and enhancement effects on quality characteristics of beef strip steaks. J. Food Sci. 70(3):S242-S248.

APPENDIX A –Figures Associated with Chapters 2

Figure A.1 Packaging treatment x day Warner-Bratzler shear force means for longissimus lumborum steaks packaged in different atmospheres
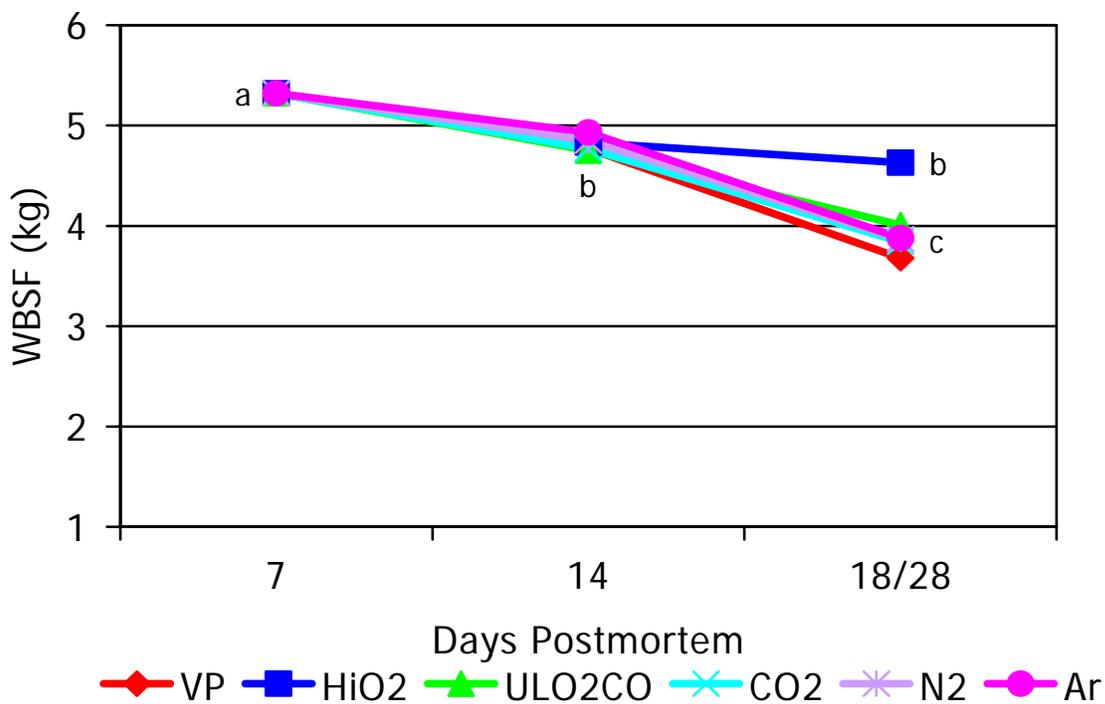

abc Means with different letters differ $P < 0.05$

Figure A.2 Display color score (2=bright red or pinkish red, 3=dull red or pinkish red, 4=slightly dark red or pinkish red, 5=reddish tan or pinkish tan, 6=moderately dark red or reddish tan or pinkish red or pinkish tan) means for longissimus lumborum steaks packaged in ULO$_2$COAr (99.6% Ar, 0.4% CO); ULO$_2$COCO$_2$ (99.6% CO$_2$, 0.4% CO); HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); ULO$_2$CON$_2$ (99.6% N$_2$, 0.4% CO); or VP (vacuum packaging; 2=bright purplish red or pink, 3=dull purplish red or pink, 4=slightly dark purplish red or purplish pink, 5=purplish tan or pinkish tan, 6=moderately dark purplish red or pink) and displayed
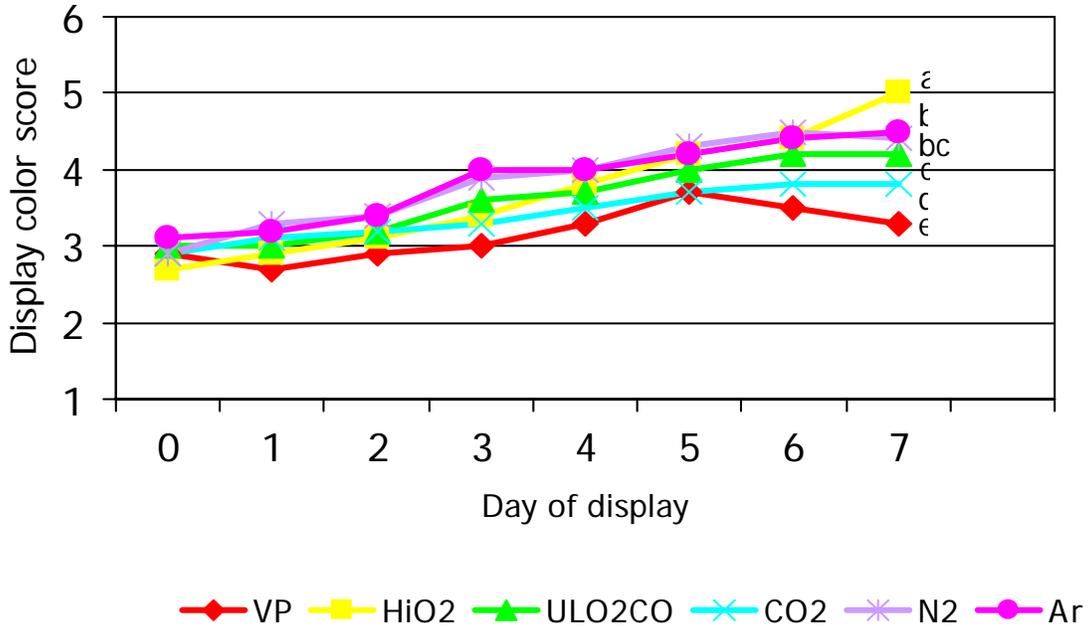

abcde Means with different letters differ *P* < 0.05

Figure A.3 Discoloration score (1= 0%, 2=1-19%, 3=20-39% metmyoglobin) means for longissimus lumborum steaks packaged in ULO$_2$COAr (99.6% Ar, 0.4% CO); ULO$_2$COCO$_2$ (99.6% CO$_2$, 0.4% CO); HiO$_2$ (80% O2, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); ULO$_2$CON$_2$ (99.6% N$_2$, 0.4% CO); or VP (vacuum packaging) and displayed
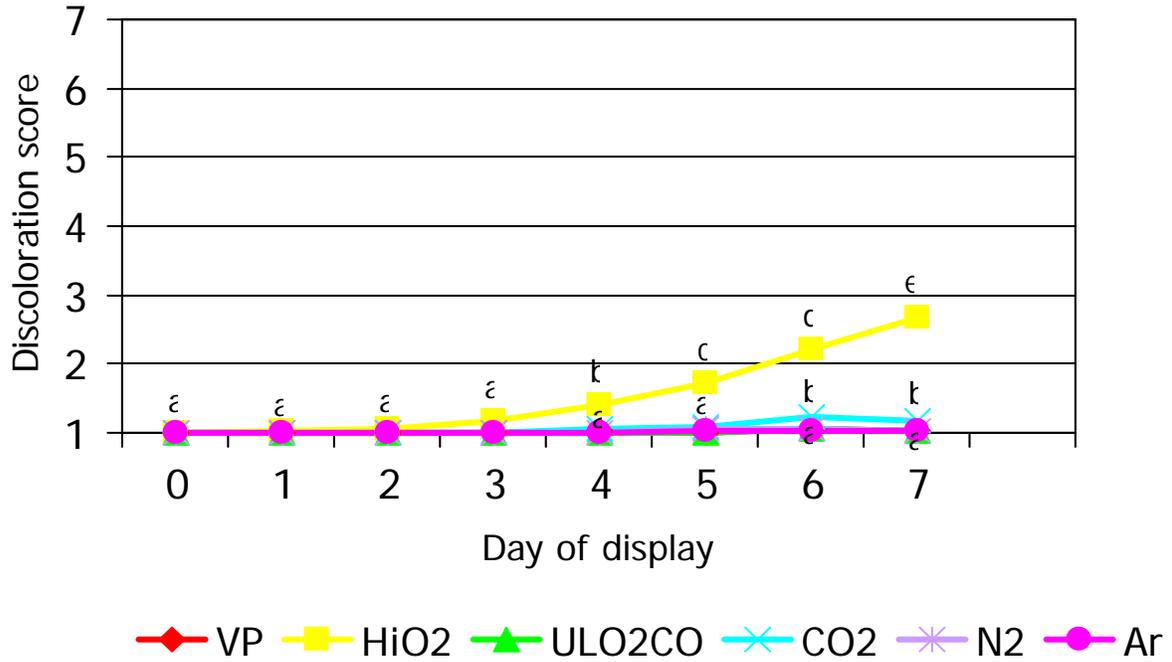

abcde Means with different letters differ $P < 0.05$

Figure A.4 Pictures from longissimus lumborum steaks on d 0, 4, and 7 of display in vacuum packaging (A); High-oxygen MAP (80% O_2, 20% CO_2; B); Ultra-low oxygen with CO (64.6% N_2, 35% CO_2, 0.4% CO; C); ULO_2COCO_2 (99.6% CO_2, 0.4% CO; D); ULO_2CON_2 (99.6% N_2, 0.4% CO; E); or ULO_2COAr (99.6% Ar, 0.4% CO; F)

d 0 of display   d 4 of display   d 7 of display
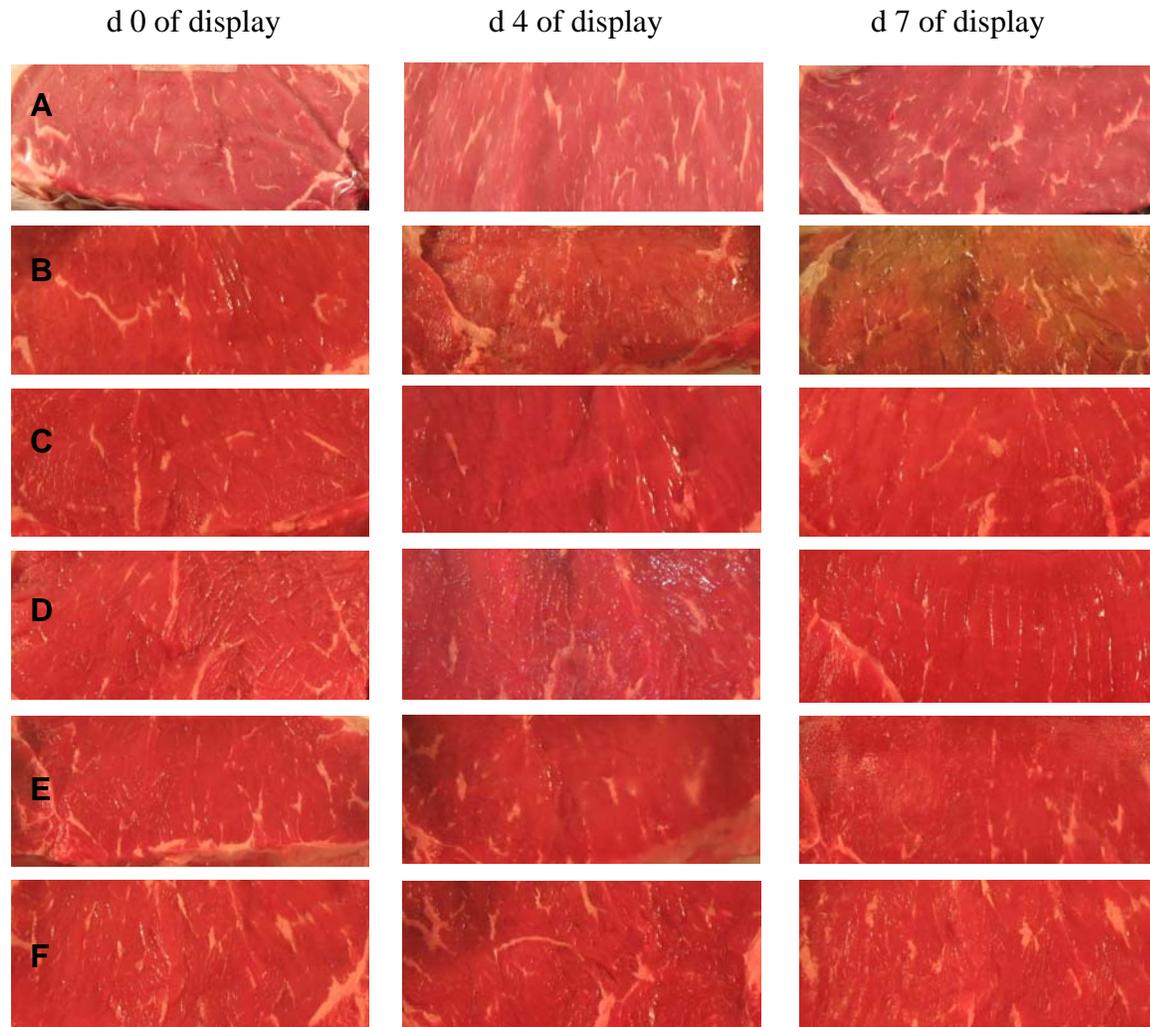
APPENDIX B – Figures Associated with Chapter 3

Figure B.1 Packaging treatment x day Warner-Bratzler shear force means for longissimus lumborum , semitendinosus, and triceps brachii steaks packaged in different atmospheres
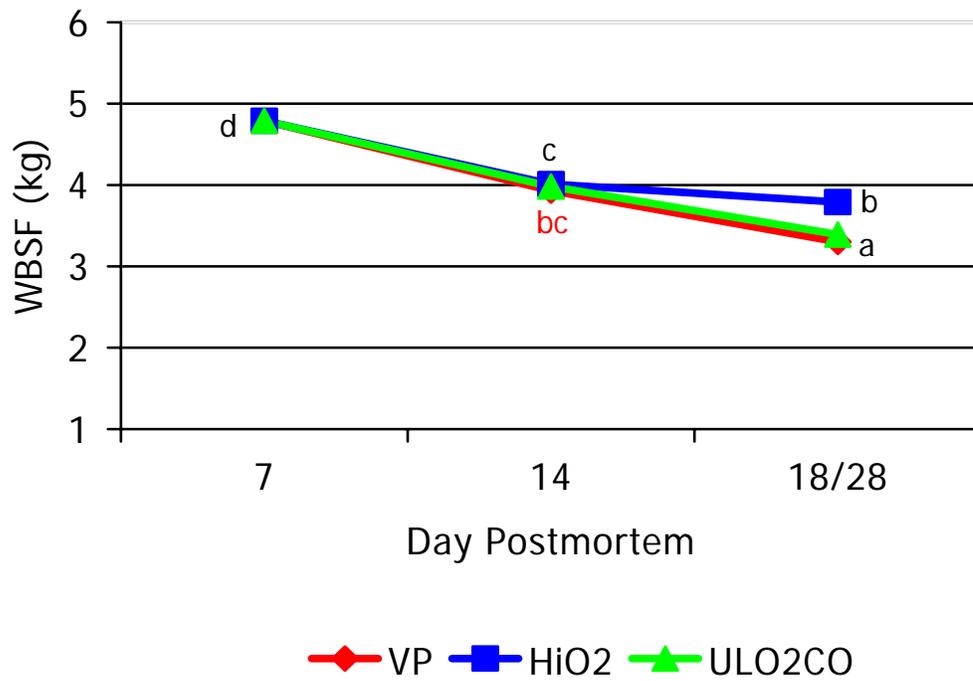
abcd Means with different letters differ $P < 0.05$

Figure B.2 Muscle x enhancement x d Warner-Bratzler shear force means for longissimus lumborum (LL), semitendinosus (ST), and triceps brachii (TB) steaks
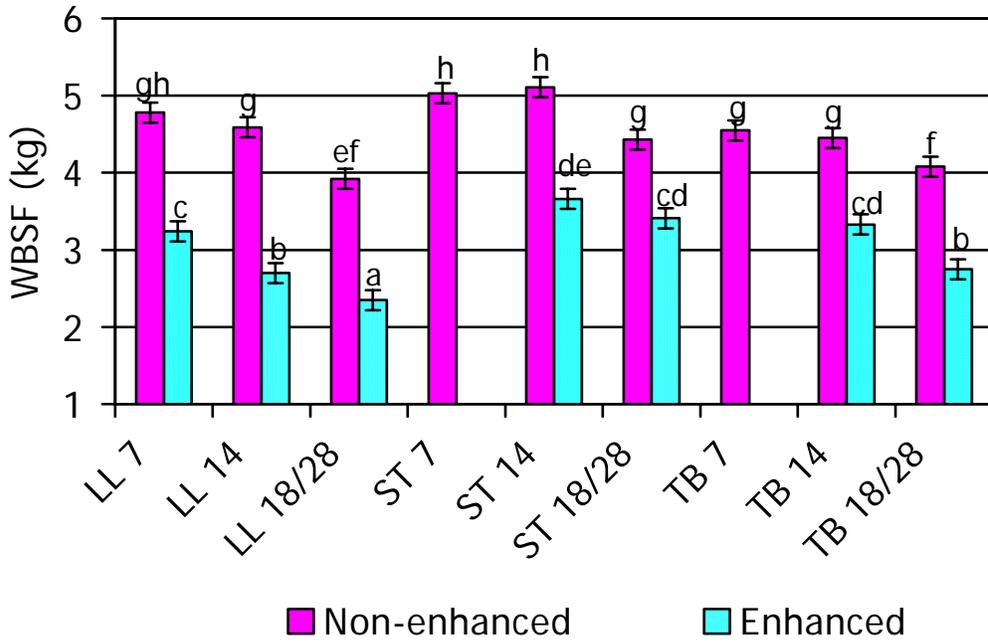
abcdefgh Means with different letters differ $P < 0.05$

Figure B.3 Enhancement x packaging treatment myofibrillar tenderness and overall tenderness means for longissimus lumborum, semitendinosus, and triceps brachii steaks
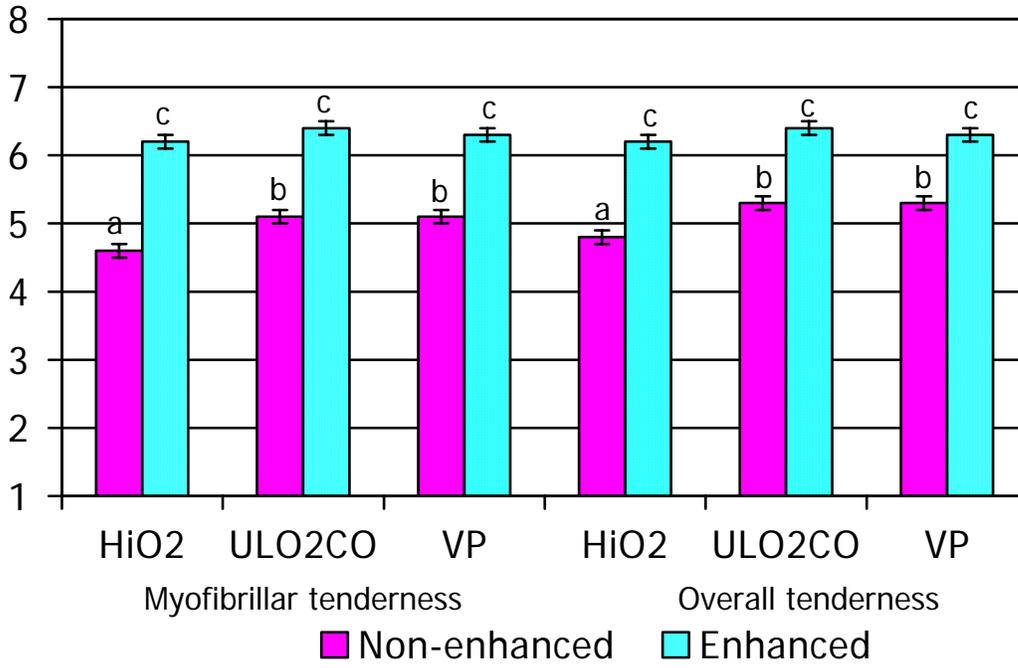
abc Means with different letters within a trait differ $P < 0.05$

Figure B.4 Enhancement x packaging treatment beef flavor and off-flavor means for longissimus lumborum, semitendinosus, and triceps brachii steaks
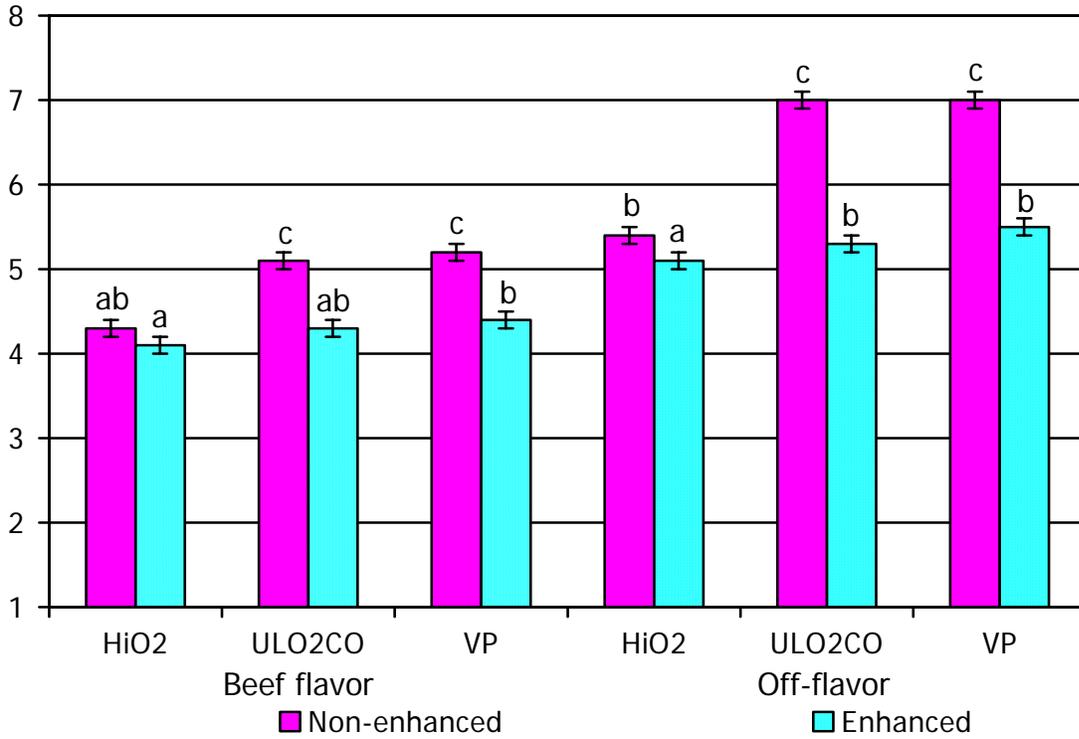
abc Means with different letters within a trait differ $P < 0.05$

Figure B.5 Muscle x enhancement beef flavor and off-flavor means for longissimus lumborum (LL), semitendinosus (ST), and triceps brachii (TB) steaks
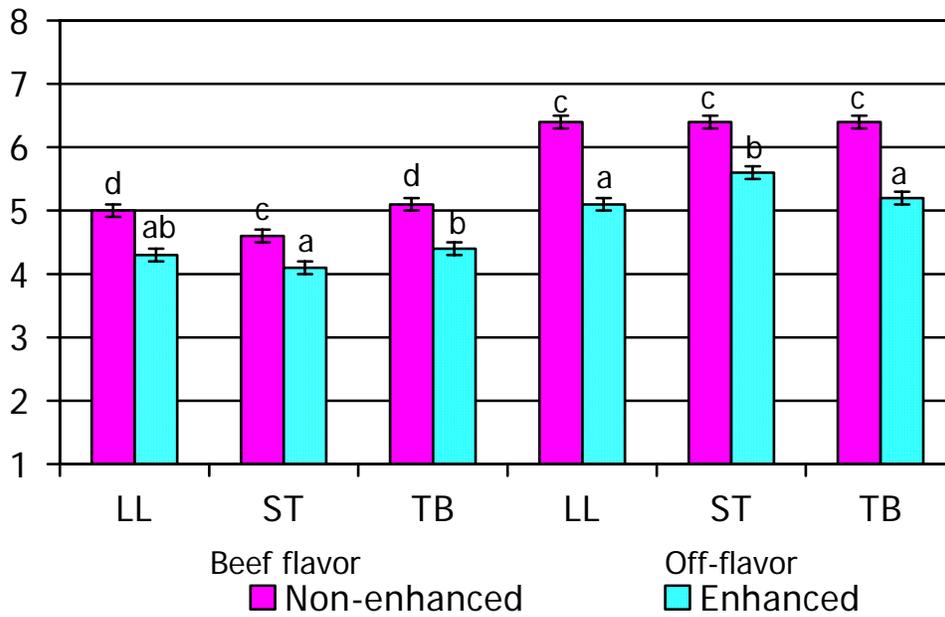
abcd Means with different letters within a trait differ $P < 0.05$

Figure B.6 Muscle x enhancement(d) desmin degradation means for longissimus lumborum (LL), semitendinosus (ST), and triceps brachii (TB) steaks
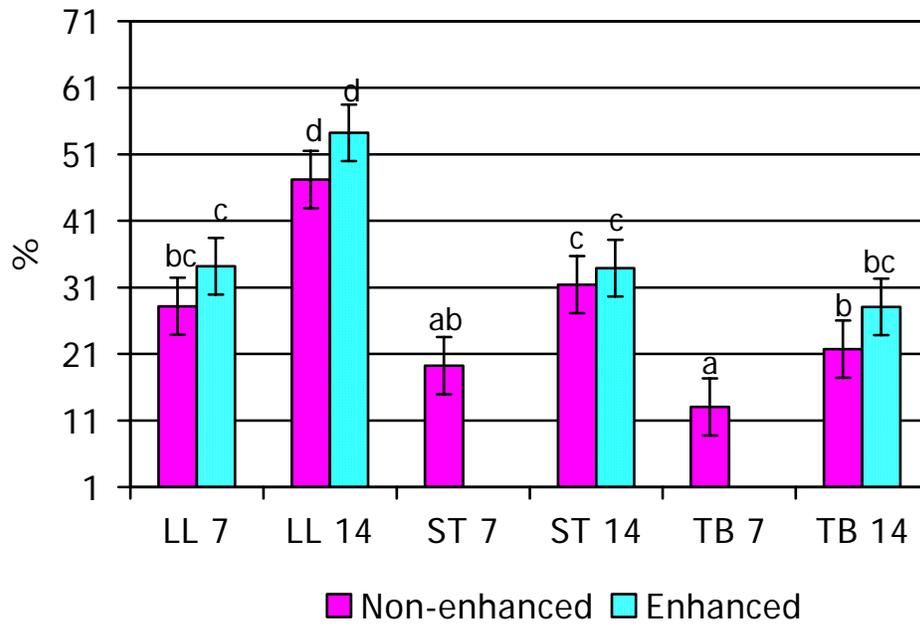
abcd Means with different letters differ *P* < 0.05

Figure B.7 Display color score means for non-enhanced (NoEnh) or enhanced (Enh) longissimus (LL; A), semitendinosus (ST; B), and triceps brachii (TB; C) steaks packaged in HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); or VP (vacuum packaging) and displayed

B

Visual color score vs d of display

- ST NoEnh HiO2
- ST NoEnh ULO2CO
- ST NoEnh VP
- ST Enh HiO2
- ST Enh ULO2CO
- ST Enh VP

c

Visual color score vs. d of display

Legend:
- TB NoEnh HiO2
- TB NoEnh ULO2CO
- TB NoEnh VP
- TB Enh HiO2
- TB Enh ULO2CO
- TB Enh VP
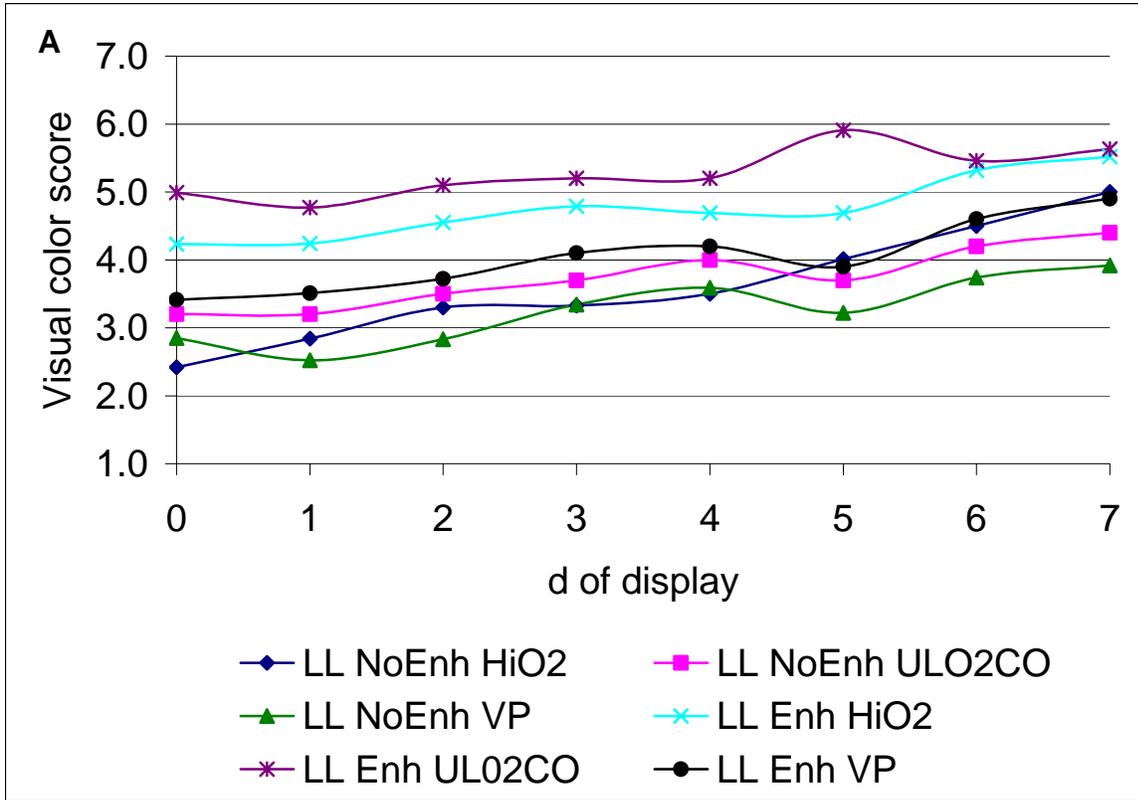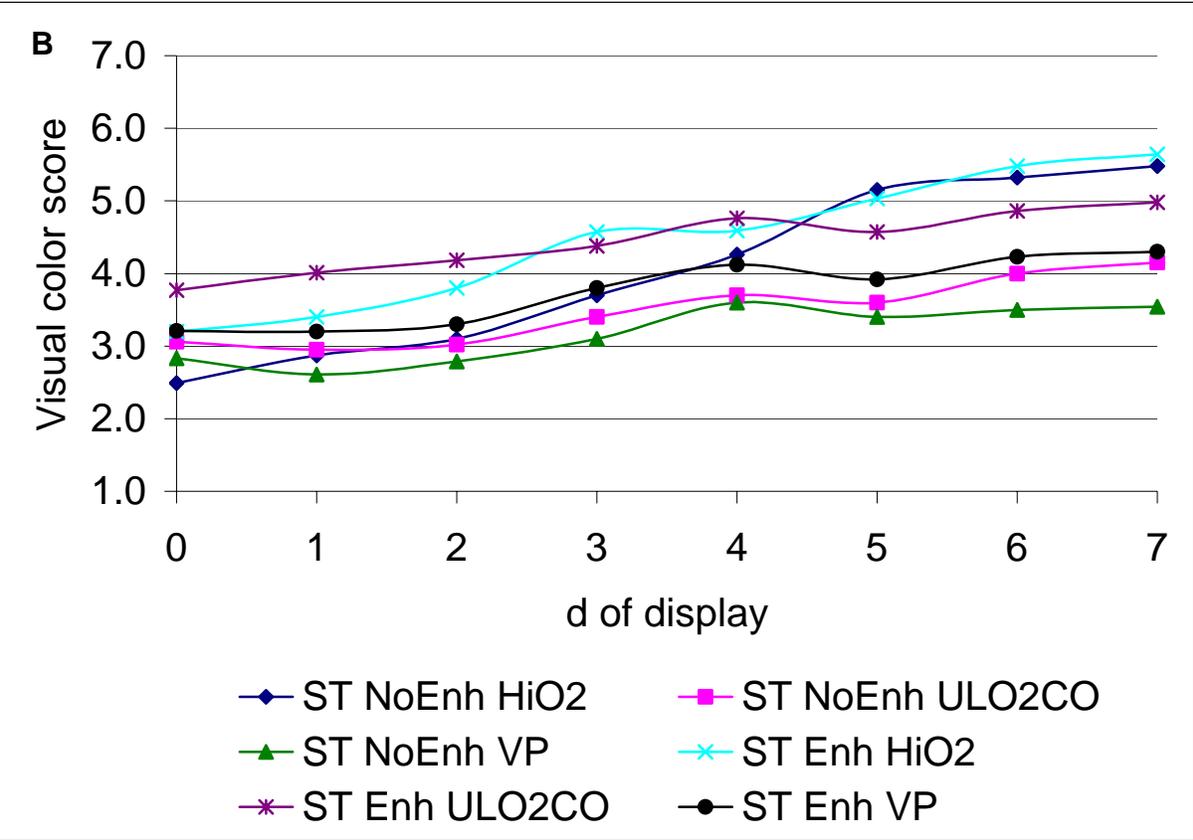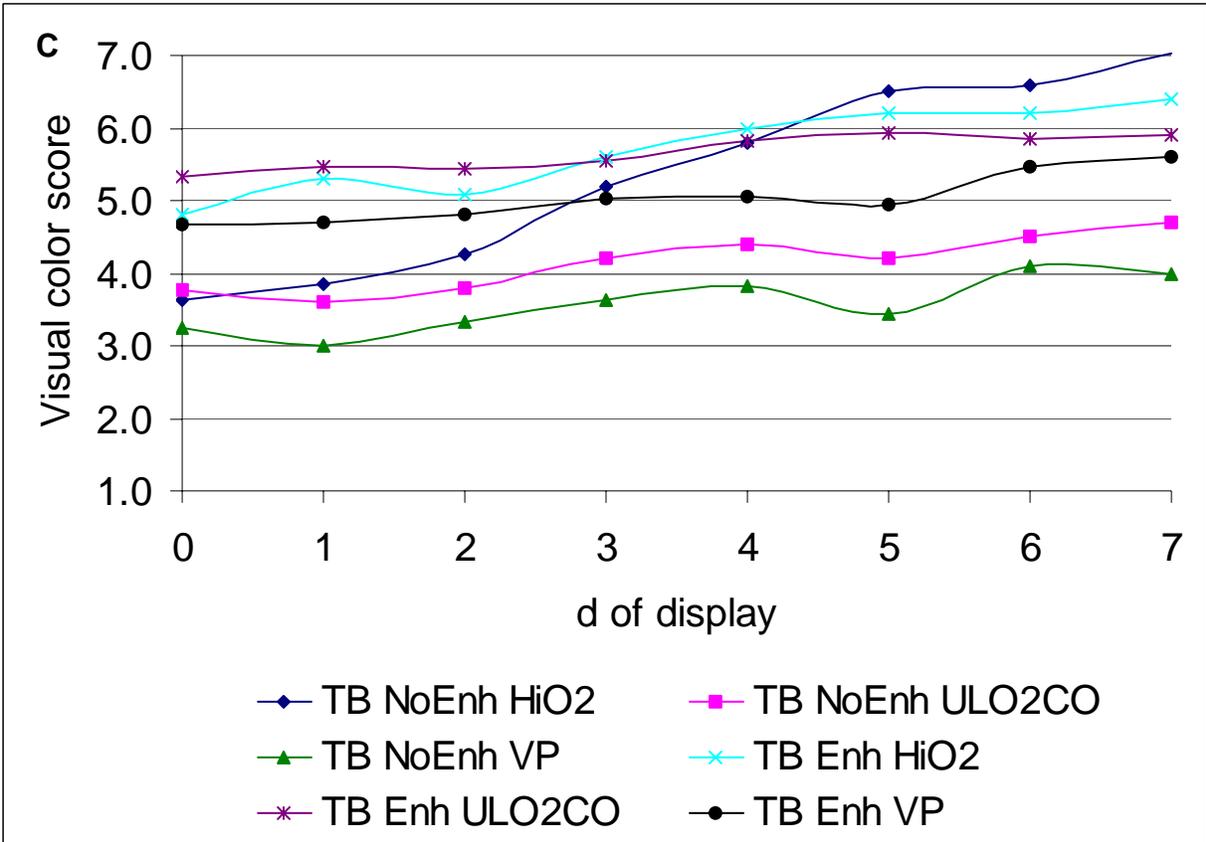
**Figure B.8 Discoloration score means for non-enhanced (NoEnh) or enhanced (Enh) longissimus (LL), semitendinosus (ST), and triceps brachii (TB) steaks packaged in HiO$_2$ (80% O$_2$, 20% CO$_2$); ULO$_2$CO (64.6% N$_2$, 35% CO$_2$, 0.4% CO); or VP (vacuum packaging) and displayed**
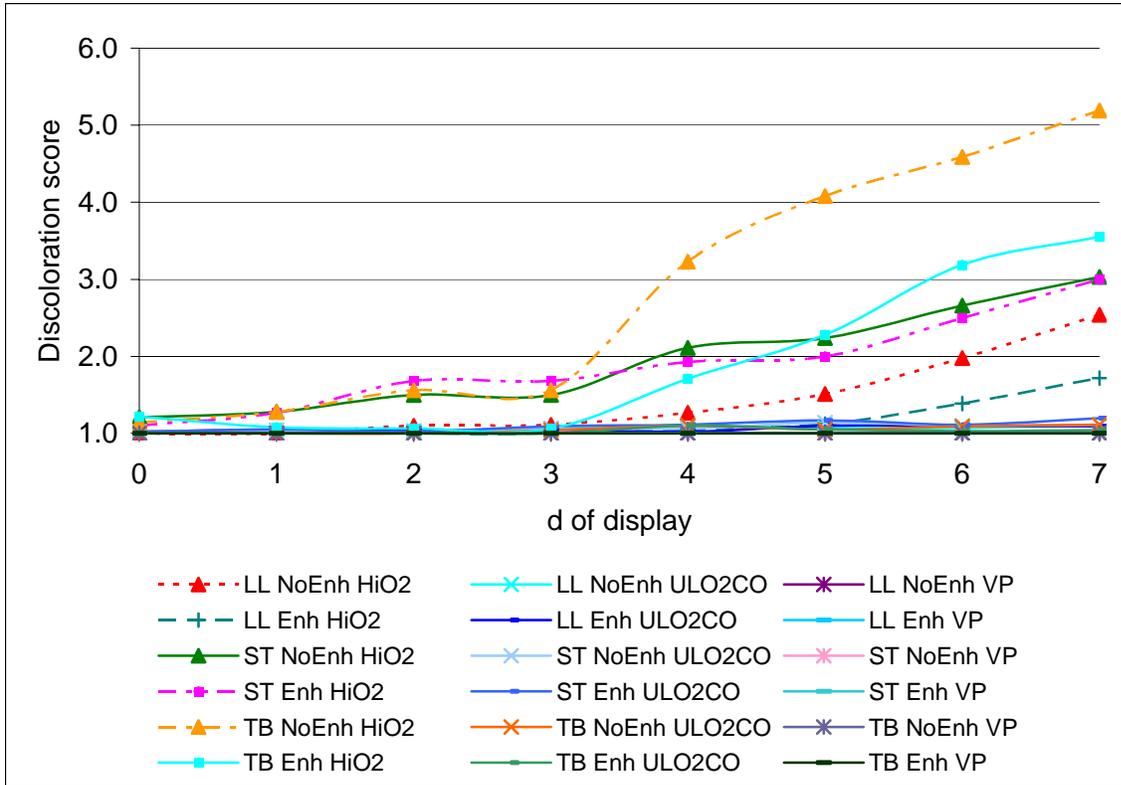
APPENDIX C – Protocol for Determining Desmin Degradation

METHOD USED TO EXTRACT TOTAL MUSCLE PROTEIN FOR WESTERN BLOT USING TRIS-EDTA BUFFER

SOLUTIONS FOR SAMPLE EXTRACTION

1. **Tris-EDTA Buffer, pH 8.3**

	1 L
50 mM Tris	6.06 g
10 mM EDTA	3.72 g

   Adjust pH to 8.3; qs t o1 liter. Store at 4°C.

2. **0.5 M Tris, pH 6.8**

	200 mL
Tris	12 g

   Adjust pH to 6.8 with HCl; qs to 200 mL. Filter and store at 4°C.

3. **10% SDS**

	500 mL
SDS	50 g

   qs to 500 mL. Filter with Whatman filter paper. Store at room temperature.

4. **Filtered Distilled Water**

5. **8 mg/ml Bromophenol Blue (0.8%)**

6. **2X Treatment Buffer minus MCE, pH 6.8**

0.125 M Tris	2.5 mlL solution (2)	50 mL solution (2)
4% SDS	4.0 mL solution (3)	80 mL solution (3)
20% glycerol	2.0 mL	40 mL
10% MCE	---	---
H_2O	0.5 mL	10 mL
	9.0 mL	180 mL

   Initial pH 7.1. pH to 6.8. Store at room temperature.

7. **2X Treatment Buffer + MCE 8 Bromophenol Blue, pH 6.8**
   9.0 mL 2X Treatment Buffer (Solution 6)
   50 µL MCE
   500 µL Bromeophenol Blue (Solution 5)

   Make fresh daily (or use aliquots that have been frozen). Any solution not used may be aliquoted and frozen for further use.

SOLUTIONS FOR GEL ELECTROPHORESIS

8. **Stock Acrylamide (30% (37.5:1)**

	100 mL
Acrylamide	29.2 g
Bisacrylamide	0.779 g

   Mix (wrap beaker in foil to prevent solution from light) and adjust to 100 mL. Filter and store in a dark bottle at 4°C.

   Caution: Acrylamide is a neurotoxin. Wear gloves and face mask when working with it. Wash hands thoroughly after use. Polymerized gels can be disposed in the trash. Unpolymerized solutions are disposed as hazardous waste, contact disposal.

9. **1.5 M Tris base, pH 8.8**

   18.15 g/100 mL ddH$_2$O   90.75 g/500 mL ddH$_2$O

   pH to 8.8 with HCl. Filter and store at 4°C.

10. **0.5 M Tris base, pH 6.8**

    6 g/100 mL ddH$_2$O   30 g/500 mL ddH$_2$O

    pH to 6.8 with HCl. Filter and store at 4°C.

11. **10% SDS**

    10 g/100 mL ddH$_2$O

    Filter (with filter paper) and store at room temperature up to 6 months. Some heat may be required to dissolve. Wear a face mask when preparing this solution.

12. **10% Ammonium Persulfate**

   1g/10 mL ddH$_2$O

M. Koohmaraie's lab prepares this and stores it in a dark bottle at 4°C.

13. **Running Buffer, pH 8.3**

	10X
0.25 M Tris (F.W. 121.1)	30.0 g
1.92 M glycine	144.0 g
ddH$_2$O to 1 L	

It is not necessary to check the pH of this solution. Store at room temperature.

To make 1X Running Buffer:	100 mL of 10X solution
	10 mL of 10% SDS
	890 mL ddH$_2$O

Make fresh for each gel run. Do not reuse the running buffer. Reusing the buffer can affect reproducibility since the ionic strength and pH of the buffer will change during the run (per Bio-Rad).

14. **Water Saturated Butanol**

   50 mL n-Butanol + 10 mL ddH$_2$O

Dispose excess as hazardous waste. Butanol that is used as gel overlay (approx. 1 mL) may be poured onto a paper towel and tehn allowed to evaporate in the hood.

SOLUTIONS FOR PROTEIN TRANSFER

15. **Transfer Buffer**

	2L	4L
Glycine	28.83 g	57.66 g
Tris	6.06 g	12.12 g
10% Methanol	200 mL	400 mL
ddH$_2$O	to 2 L	to 4 L

Should be pH 8.1 – 8.3 without pHing. This solution may be reused 1-2 times. Dispose in sewer system.

16. **0.1% Amido Black Staining Solution**

	100 mL
Amido Black	100 mg
10% Methanol	10 mL
2% Acetic Acid	2 mL
ddH$_2$O	88 mL

Mix and filter. Store at room temperature. Dispose in sewer system.

17. **Destain**

20% Methanol	2000 mL
7 % Acetic Acid	700 mL
ddH$_2$O	7300 mL

Dispose in methanol hazardous waste container.

SOLUTIONS FOR WERSTERN BLOT

18. **TBS, pH 7.4**

	1 L	or	10X	1 L
20 mM Tris	2.4 g			24 g
137 mM NaCl	8.0 g			80 g
5 mM KCl	0.2 g			2 g
				(dilute to 1X to use)

pH with 1 N HCl. Filter and store at room temperature.

19. **TTBS (0.05% Tween 20), pH 7.4**

Add 250 µL Tween 20 to 500 mL TBS.

20. **Blocking Solution**

2.5% Sheep Serum	12.5 mL of crude prep sheep serum
TTBS	500 mL

SAMPLE PREPARATION

1. Homogenize 1 g of sample in 10 volumes (10 mL) 1X extraction buffer (Solution 1) for 20 sec with the Polytron at setting #4. Do this step in the cold room if using raw samples. For cooked core samples, this step may be done in the lab.

2. <u>Immediately</u> remove a 0.5 mL aliquot for solubilization and transfer to a 1.5 mL microcentrifuge tube.

3. Add 0.5 mL of 2X Treatment buffer (minus MCE). Mix well by repeatedly pipetting. Nucleic acids may be stringy and viscous, but pipetting will help shear them. Vortex sample.

4. Heat samples in a 50°C water bath for 20 minutes, repeat mixing, and reheat for 5 min.

5. Centrifuge for 20 min in a Eppendorf 5414 C centrifuge (maximum setting = 16,000 x g), to pellet insoluble material. Pellet should be small or undetectable.

6. Determine protein concentration of the supernatant (diluted 1:5 with 1X Treatment Buffer (10 µL sample + 40 µL buffer) using the micro-BCA protein assay (use microtiter well plates). Do in triplicate.

    To each well add:

Sample:	10 µL diluted sample
or	
Standard:	10 µL (4, 2, 1, 0.5, 0 mg/mL BSA)

    Add 200 µL BCA reagent and incubate at 37°C for 30 min. Read plate on the microplate reader at 562 nm. If more than one plate is used, run a standard curve with each plate. Mix standards to contain the same concentration of potential interfering substances as the samples.

7. Dilute samples to 3 mg/mL (or your desired protein concentration) using 2X treatment buffer containing MCE and bromophenol blue (Solution 7). Mix samples well and heat in a 50°C waterbath for 10 min prior to loading on gel. Samples may be frozen at this point if gels cannot be run at this time.

GEL ELECTROPHORESIS

1. The height of the separating gel is 5.5 cm. A 4% stacker is used.

	4% Stacker	SEPARATING				
		15%	12.5%	10%	7.5%	5% Continuous
1.5 M Tris, pH 8.8	---	5.0 mL	5.0 mL	5.0 mL	5.0 mL	5.0 mL
0.5 M Tris, pH 6.8	1.88 mL	---	---	---	---	---
30% Acrylamide	1.0 mL	9.98 mL	8.35 mL	6.65 mL	4.99 mL	3.3 mL
10% SDS	0.075 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL
Filterd ddH$_2$O	4.55 mL	4.72 mL	6.35 mL	8.05 mL	9.71 mL	11.2 mL
10% APS	50 µL	100 µL	100 µL	100 µL	100 µL	100 µL
TEMED	7.5 µL	10 µL	10 µL	10 µL	10 µL	10 µL

2. Mix separating gel and degas 15 min. Add TEMED.

3. Pour gel (5.5 cm); overlay with water saturated Butanol and allow to polymerize 1 hr.

4. Make stacking gel and degas 15 min. Add APS and TEMED and mix immediately before stacker is to be poured (see step 5).

5. Pour off water saturated Butanol and rinse well with distilled water. Remove any residual water with a Kimwipe. Pour stacker making sure that no air bubbles are trapped under the wells. Place comb in between plates. Allow to polymerize 30-45 min.

6. Carefully remove comb and rinse wells with water. Remove residual water with a Kimwipe. Assemble gel rig using running buffer in the lower chamber and running buffer in the upper chamber.

7. If samples were frozen, heat thawed samples in a 50°C water bath for 5 min.

8. Run gels at 200 volts for 45 min or until dye front just runs off the end of the gel.

9. While gel is running, prepare everything to transfer proteins from the gel to the membranes.

SAMPLE LOADING

1. A standard is run on every gel in triplicates. The standard preferably is a pooled sample of multiple animals collected at 0 h postmortem. Based on the samples to be run, the standard must be species and muscle specific.

2. Always leave one or two outside lanes open (to avoid sample smiling). The standard is run in the outside lanes and the middle lane (see example).

Lane	
1	PDB
2	**0 h standard**
3	Sample
4	Sample
5	Sample
6	Sample
7	Sample
8	**0 h standard**
9	Sample
10	Sample
11	Sample
12	Sample
13	Sample
14	**0 h standard**
15	PDB

PROTEIN TRANSFER

1. Cut blotter paper (Whatman 3MMChr Chromatography paper) to 3 in x 4 in. Cut the PVD Membranes to 2.5 in x 3.5 in. Notch the upper left corner of the membrane. This notch will correspond to lane 1 of the gel. Using a pencil, mark your ID in this corner. Be careful to never touch the membranes with your hands – always wear gloves. Handle membranes with forceps.

2. Place transfer buffer in a tray. Assembling of the sandwiches will take place in this tray. Lay 1.2 of the plastic cassette in the tray. Place 1 buffer saturated sponge on top of this.

3. Remove one gel from rig and remove one glass plate. Remove <u>all</u> stacker. You may have to rub the glass plate with your finger to insure that all the stacker has been removed. Notch the gel at lane 1. Slide the gel into the tray containing transfer buffer.

4. Wet blotter paper (2 sets, 2 papers each) in transfer buffer.

5. Fill one tray (pipet tip box lids) with methanol and another with distilled water. Wet one membrane in methanol for approximately 5 sec, making certain that the membrane is totally submerged. Transfer this membrane to the tray containing water for 30 sec. Transfer to Transfer Buffer.

6. Transfer Stack Assembly. The sandwich is assembled so that the negative charge travels through the gel to the membrane. Assemble as follows: On top of the saturated sponge place 2 buffer saturated blotter papers. Remove air bubbles by rolling a 15 mL conical over the surface. Place the membrane on top of the blotter paper, curved side up. Make sure there are no air bubbles under the membrane. Center the gel on tip of the membrane. Gently remove air bubbles with your finger. Make certain your gloves are wet or the gel will tear. Place 2 buffer saturated blotter papers on the gel, from the center towards the edge. Roll a 15 mL conical over the paper surface to remove air bubbles. During this process, keep all surfaces wet. Place 1 buffer saturated sponge on top of the blotter paper. Place a plastic cassette on top of this. Place sandwich in tank with the gel on the negative electrode side (black = negative; red = positive). Fill tank with cold Transfer Buffer. Transfer is done at 4°C. Place the tank on a stir plate with gentle stirring.

7. Transfer. 10% gels transferred at 200 mA for 1 h at 4°C

8. Rinse blot in water for about 10 sec and place in blocking solution and store overnight at 4°C.

WESTERN BLOTTING PROTOCOL FOR PIERCE SUPERSIGNAL WEST DURA EXTENDED DURATION SUBSTRATE

10 mL sufficiently covers 1 membrane.

1. Perform electrophoresis and transfer as listed in the protocol, Method Used to Extract Total Muscle Protein for Western Blot Using Tris-EDTA Buffer.

2. If blot was air dried, re-wet blot in Methanol and then water.

3. Non-specific binding sites are blocked by immersing the membrane in TTBS + 2.5 Sheep Serum for 1 h at room temperature on an orbital shaker. Membranes may be left in the blocking solution overnight in a refrigerator.

4. During the blocking step, dilute the primary antibody in TTBS. (1:300 D_3 specific for desmin)

5. Incubate the membrane in diluted primary antibody for 1 h at room temperature.

6. Filtered TTBS (0.05% Tween) is used for the washes. Using a squirt bottle filled with TTBS, briefly rinse the membrane using the 2 changes of TTBS. Wash once for 15 min and twice for 5 min with fresh changes of TTBS at room temperature.

7. During the washing step, dilute the secondary antibody in TTBS (1:10,000 anti-mouse). Use the Pierce ECL antibody anti-mouse.

8. Incubate the membrane in diluted secondary antibody for 1 h at room temperature.

9. Using 20 mL/membrane, wash the membrane 2 x 15 min and 4 x 5 min in fresh changes of TTBS.

10. Detection.

DETECTION

It is necessary to work quickly once the membranes have been exposed to the detection solution.

1. Using the Pierce SuperSignal West Dura Extended Duration Substrate Kit, mix an equal volume of West Dura Lumino/Enhancer solution with West Dura Stable Peroxide Solution to give sufficient coverage of the membrane. 3.0 mL will cover one membrane. This substrate can be reused twice without significant loss of signal.

2. Place membrane in a tray, protein side up.

3. Add the detection solution and incubate for 5 min at room temperature without shaking.

4. Drain off excess detection reagent and transfer membrane to a sheet protector. Gently smooth out air pockets.

5. Place the membrane, protein side up, in the light box. Adjust the camera for enlargement, focus, and light.

6. Expose the membranes for the desired time (5 min). Check image exposure saturation after first exposure. If saturated, reduce exposure time and re-expose blot. Desired exposure time is reflective of minimal or no saturation across all bands on the blot.

7. Save the images after exposure is complete.

DATA ANALYSIS USING THE ALPHA INNOTECH IMAGE ANALYSIS SYSTEM

1. QUANTIFICAITON: Using the Spot Density function, draw tight boxes around the bands of interest. Do <u>not</u> use the auto background function. Do not use the enhance function unless it is a picture being used for publication purposes.

2. CALCULATIONS: Obtain the IDV mean for all three 0 h standard samples. Calculate the % desmin remaining using the formula: (protein IDV / mean of pooled 0 h standard IDV) x 100

OPTIMAL CONDITIONS FOR ECL ANTIBODY DETECTION PIERCE SUPER SIGNAL WEST DURA EXTENDED DURATION SUBSTRATE FOR DESMIN – D3

> 10% gel (30% acrylamide, 37.5:1)
> Transfer 1 h at 4°C at 200 mA
> Blocking agent: TTBS + 2.5% Sheep Serum
> Protein concentration: 15 µg (15 well gel)
> Primary dilution: 1:10 (will vary for each lot of D3)
> Secondary dilution: 1:10,000 Pierce antibody, anti-mouse
> ECL detection:
>> Incubation: Pierce West Dura Substrate; 5 min incubation
>> Exposure: 5 min exposure