EFFECT OF OXYGEN EXPOSURE ON COLOR STABILITY OF GROUND BEEF

by

DORLY PISKE

B.S. Federal University of Santa Catarina, Brazil, 1976

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

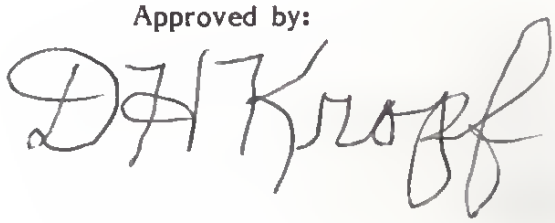
Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Approved by:



To

my family

teachers

friends

Richard

for what they mean to me

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INTRODUCTION

Ground beef shares an important portion of the fresh beef market in the USA, and is largely sold in the bright red form. To facilitate longer storage and/or display life and shipping long distances, ground beef trim generated at fabrication plant is often coarse ground and stuffed into an oxygen permeable casing. This is then fine ground at the retail store and should form the bright red color upon exposure to air. Failure of development of the bright red color or poor color stability (short shelf life) has been reported in the industry and represents product loss.

Understanding and control of the factors that may extend color stability during display in oxygen permeable packages at the retail stores is important and should help to reduce product loss.

The purpose of this study was to evaluate the effect of minimized exposure to air during processing of ground beef on the color stability.

Chapter I

REVIEW OF LITERATURE

Structure and Properties of Myoglobin

Meat color is largely determined by muscle structure and by concentration and chemical state of a complex and relatively unstable protein, myoglobin (Mb). Mb concentration is dependent on muscle function and location. Relative proportions of the three major chemical states of myoglobin; oxymyoglobin (oxyMb), deoxymyoglobin (deoxyMb) and metmyoglobin or ferric myoglobin (metMb) are dependent on oxidative-reductive state and on the partial oxygen pressure (pO_2). These pigment states are in a state of dynamic equilibrium depending on conditions at individual locations in the muscle.

Mb structure and reactivity is affected by muscle changes after slaughter and during processing, storage and distribution (Giddings, 1977). Changes in pH affect the morphology of muscle; consequently the light scattering/absorbing properties (Solberg, 1970).

Although Mb is the major component responsible for fresh meat color, and in most studies it is used alone as an index of color, hemoglobin constitutes as much as 12 to 30 % of the total pigment (Rickansrud and Henrickson, 1967). However, Bunnig and Hamm (1974) found that the hemoglobin content of meat from well-bled animals was less than 6 %. The earlier higher figures were attributed to methodology limitations and consequently overestimation of hemoglobin. The spectral properties and chemical reactions of both pigments are similar so that any spectral color measurement also includes the contribution of hemoglobin (Govindarajan, 1973).

Mb is associated with the sarcoplasmic protein fraction of muscle that is soluble in water and dilute salt solutions. It has vital functions of supplying oxygen for muscle metabolic activity (tissue respiration) in living animals. Mb may be localized very close to mitochondria to maintain desirable intracellular pO_2 (Giddings, 1974).

Mb is contained mainly in cardiac and skeletal muscles and is present in relatively high concentrations in the "red", Type I, oxidative, slow twitch, tonic fibers, to a lesser extent in the "intermediate" fibers and is virtually absent in "white", Type II, glycolytic, fast twitch, phasic fibers (Giddings, 1974). Mb is a monomeric, globular heme protein. The single heme moiety has one iron atom per molecule, and has a molecular weight of about 18,000. Hemoglobin, by comparison, is a tetrameric globular heme protein containing four heme structures (M.W. about 67,000), and is localized in the erythrocytes. The polypeptide chain of Mb is folded into helical segments in a manner to give the molecule a flattened, globular shape. The heme group is "trapped" inside the essentially hydrophobic globin in a precise geometric orientation, protected from solvents and contacted by approximately 25 amino acid residues of the protein. Hydrogen bonds, salt bridges and hydrophobic interactions stabilize the native conformation of the protein. The only covalent bond between the heme and protein moieties is that of the iron ligand to the histidine group which plays an important role in oxygen binding (Livingston and Brown, 1981).

The oxidation state and type of ligand bound to the iron determine the color and reactivity of Mb. The heme iron atom has eight valence electrons; because of its low electronegativity it may form ferrous (Fe^{2+}) or ferric (Fe^{3+}) cations by losing 2 or 3 valence electrons, respectively. Both Fe^{2+} and Fe^{3+} usually have 6 ligands bound to them, except native Mb which has 5. Four of these

positions are occupied by the heme pyrrole nitrogens forming a very stable chelate (Livingston and Brown, 1981). The fifth position (axial or proximal) binds histidine. The sixth position is left open for substitution by oxygen or other ligands or is liganded and hydrogen bonded to the distal histidine. The O_2 molecule, with two impaired electrons, bonds to the Fe^{2+} but not to the Fe^{3+} (Solberg, 1970).

In native Mb, only small molecules can bind to the iron because of steric hindrances. If denaturation occurs, coordination with larger ligands can occur. The series of changes that muscle undergoes after slaughter include depletion of oxygen, pH and temperature decline, increased cell membrane permeability, drop in redox potential, lower and altered enzymatic activities and are, therefore, important factors influencing fresh meat color (Solberg, 1970). Conditions describing the three forms of Mb in meat are summarized on Table 1.

Deoxymyoglobin, the deoxygenated, reduced form of Mb is the native meat pigment. Its iron atom is in the ferrous state and has no ligand between it and the distal histidine residue (sixth position). It is responsible for the purplish-red color found in the interior of freshly cut meat.

Partial pressure of oxygen (pO_2) is the major external factor, besides temperature, that affects the chemical state of myoglobin. George and Stratmann (1952b) studied this effect extensively. DeoxyMb is formed when pO_2 in muscle and meat is below 1.4 mm Hg. Oxidation begins to occur at 1.4 mm Hg and metMb dominates at this and slightly higher pO_2. As the pO_2 increases, the potential for oxidation decreases. At about 20 mm Hg this reaction levels off and a continual conversion of deoxyMb to oxidized metMb can be expected. The pO_2 of normal atmosphere is approximately 160 mm Hg.

Table 1. Description of the three forms of myoglobin molecule in meat (Solberg, 1970).

Pigment	State of iron	Ligand 6th site	Visual appearance
Deoxymyoglobin	Fe^{2+}	--	purple
Oxymyoglobin	Fe^{2+}	O_2	bright red
Metmyoglobin	Fe^{3+}	H_2O	brown

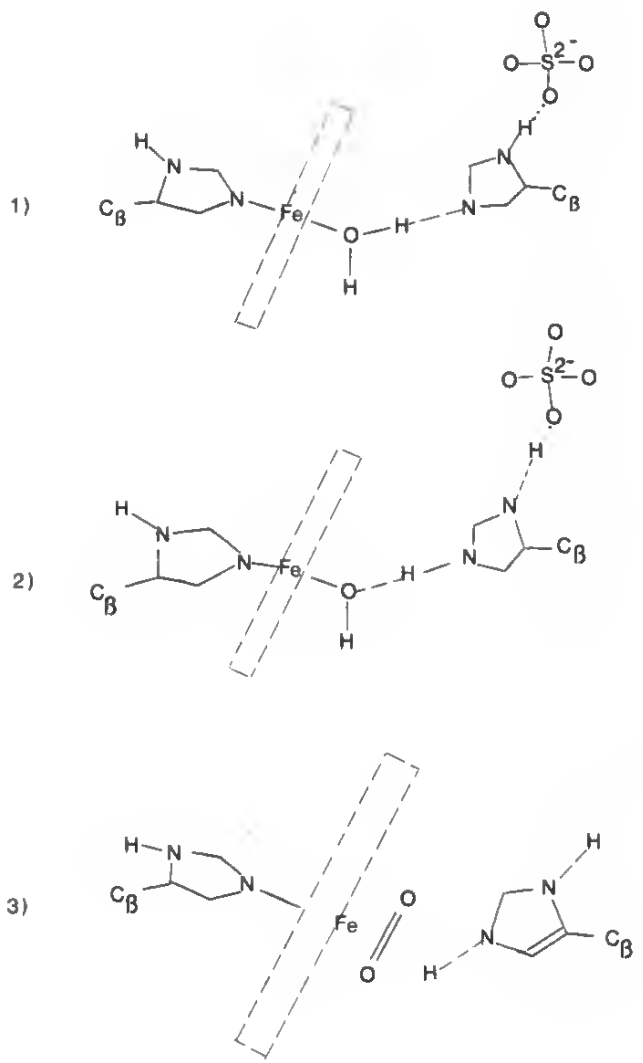

Fig. 1. Structural representation of the myoglobin states: 1) and 2) proposed for metmyoglobin and 3) oxygen binding of oxymyoglobin (Govindarajan, 1973).

Oxymyoglobin is a stable derivative under high oxygen conditions and its color is bright red. An oxygen molecule is situated parallel to the heme plane; there is a hydrogen bond of the oxygen molecule to the distal histidine and a covalent bond to the ferrous iron atom (Fig. 1, Solberg, 1970). The reaction is exothermic (ΔH -13 to -20 kcal/mol) and spontaneous, therefore the formation of oxyMb on the surface of meat exposed to air is rapid. The reaction is also reversible, but with a dissociation equilibrium constant of 2.1×10^{-6} which means the equilibrium of the reaction lies far to the left, making it much more stable to oxidation than deoxyMb. The hydrophobic environment around the heme is the suggested reason for greater stability against oxidation (Govindarajan, 1973).

Metmyoglobin has its distal histidine hydrogen bonded to a water molecule which is covalently attached to the oxidized iron atom (Fig. 1). This univalent oxidation of oxyMb is commonly referred to as "autoxidation", meaning a nonenzymatic, spontaneous oxidation by free oxygen. This expression implies that a free superoxide anion (O_2^-) is formed; but O_2^- cannot dissociate for reasons of mechanism and thermodynamics. An interpretation that better explains the observed effect of proton concentration, particularly over the pH range from 5 to 7 is that a hydroperoxy radical is dissociated from the oxyhemoprotein as one major pathway for autoxidation. A mechanism based on this compound and on oxygen uptake/evolution stoichiometry (0.25 mol per 0.75 mol) and dependent on the amount of oxygen dissolved has been reported by Brown and Mebine (1969). Another mechanism based on the findings of George and Stratman (1952a;b), and since confirmed by others, involves a two-equivalent reduction of bound oxygen to the peroxide level, with one electron donated by the iron and the second one donated by a one-equivalent reducing agent (metal ions or organic reducing agents) in intimate proximity to the ligand heme, or by electron tunneling from a redox

reaction site on the globin remote from the heme or on the periphery of heme itself. The heme-globin may be considered to be a system in a state of equilibrium. At physiological pH the association/dissociation rate is very low so autoxidation is not appreciable; but the rate is high at lower pH. This, along with globin unfolding and bound oxygen protonation accounts for the pH effect on autoxidation (Giddings, 1977).

MacDougall and Taylor (1975) demonstrated the positive effect of temperature on oxidation rate, which can be promoted when temperature of fresh meat rises. The amount of dissolved O_2 is lowered and also any remaining respiratory activity is accelerated. The well documented relationship between oxidation and the pO_2 can be accounted for by this mechanism.

George and Stratman (1952a;b) observed that while autoxidation at saturating pO_2 had a simple first-order kinetics, at much lower concentrations the observed rate constants showed a complex second-order variation according to the concentration of deoxyMb and oxyMb present.

Oxygenated heme iron contributes one electron and the uncomplexed iron of deoxyMb contributes the second (outer sphere or peripheral type mechanism). Depending on the donor compound, an inner-sphere or axial mechanism can also be involved (Giddings, 1977).

This information indicates more than one mechanism of autoxidation, which vary depending on the conditions and would increase reactive hydroxyl when a peroxide anion reacts with the ferric heme to which an oxygen had been liganded and might catalyze free radical reactions such as lipid oxidation (Giddings, 1977).

Factors Affecting Metmyoglobin Formation

Muscle location. Muscles vary greatly regarding color stability, mainly due to anatomical location, function and structure which influence the oxygen demand of the respiratory enzymatic system.

Mb is found in higher concentration in "red" fibers and is virtually absent in "white" fibers (Giddings, 1974). High Mb content in muscle is associated with high oxidative enzymatic activity, particularly cytochrome oxidase. The more Mb in skeletal muscle, the greater its capacity for oxidative metabolism and the less its capacity for glycolytic metabolism, and vice-versa (Lawrie, 1952). A high respiratory capacity in the postmortem muscle strongly affects pigment oxidation.

Billaut et al. (1984) studied the rate of discoloration of 57 different beef muscles, prepackaged and stored at 0° C. Color changes during display, measured by a visual scoring system based on a discoloration index (0 = bright red with no discoloration; 5 = 50 % of the surface discolored), largely varied with muscles. The length of storage with minimum discoloration was greater for obliquous externus abdominis, transversus abdominis and latissimus dorsi muscles; whereas less stable muscles were diaphragma medialis, iliacus, diaphragma pars lateralis, biceps femoris and adductor. A similar study (Hood, 1980) of 6 muscles measured the rate of metMb accumulation in pre-packed beef. Longissimus dorsi was the most stable, semitendinosus, semimembranosus and vastus lateralis were of intermediate stability, and gluteus medius and psoas major the least stable muscles. The author points out that from a practical point of view it is important to consider that the least stable muscles occur in the most expensive cuts and that pre-packaging techniques might increase these differences in appearance. These results correlated well with Ledward (1971), where the muscle susceptibility to metMb formation and discoloration from greatest to least was biceps femoris, semimembranosus,

longissimus dorsi and semitendinosus; and with the results of Hood (1971), where degree of metMb formation from greatest to least was psoas, gluteus medius and longissimus dorsi.

The same behavior of these muscles has been observed by O'Keeffe and Hood (1982) under a variety of storage treatments. This difference in behavior can be explained by the basic biological and biochemical properties of stable and unstable muscles. The main characteristics of psoas major and longissimus dorsi are listed on Table 2. Psoas major muscle tends to convert oxyMb to deoxyMb more rapidly beacause of rapid O_2 consumption by the oxidative enzymes and relatively low O_2 availability in the tissue because of low O_2 storage capacity by Mb. The reduced form is more readily oxidized. The high respiration rate is associated with high SDH (succinic dehydrogenase) activity.

pH. Mb oxidation was found to be directly dependent on the hydrogen ion concentration, with rate of oxidation increasing rapidly with decreasing pH (Brooks, 1931). The pH, particularly in the range 5 to 7, influences the heme-globin association/dissociation rate (George and Stratmann, 1952a;b). According to Brown and Mebine (1969), bovine Mb oxidation proceeds 2.5 times faster at pH 6.0 than at pH 6.5. Low final pH thus enhances Mb oxygenation and oxidation, the latter leading to fading or browning of color.

PSE pork color problems are related to the more heat and pH sensitive Mb (Bembers and Satterlee, 1975). With higher final pH, aerobic metabolism remains active when most of the Mb remains in the reduced state, since oxygen consumption rate at the surface is higher than in meat with a normal pH decrease (Ashmore et al., 1972).

Table 2. Comparison of biochemical properties in psoas major and longissimus dorsi muscles (O'Keeffe and Hood, 1982).

	Psoas major	Longissmus dorsi
Oxygen consumption rate (OCR)	High	Low
Oxygenation of intact muscle	Efficient	Less efficient
Oxymyoglobin layer	Narrow	Wide
Succinic dehydrogenase (SDH) activity)	High	Low
Oxidation with $K_3Fe(CN)_6$	Easy	Difficult
Myoglobin content	"Lower"	"Higher"
Conversion of oxymyoglobin to myoglobin	Rapid	Slow
Metmyoglobin formation during conversion of oxymyoglobin to myoglobin	Strong	Weak
Metmyoglobin reducing activity (MRA)	Low	High
Colour stability	Poor	Good
Aerobic reducing activity (ARA)	Low	High

In normal carcasses, glycolysis and the resulting lower pH inactivates mitochondria and associated enzyme systems. Oxygen consumption is inhibited and consequently Mb remains more fully oxygenated, resulting in bright red color. The pH of meat also influences the perceived color by affecting water binding capacity of meat proteins. If pH is closer to the isoelectric point, less water is bound and more incident light is scattered and the meat appears less red (Lawrie, 1958). More recently, Offer and Trinick (1983) suggested that differences in meat color associated with different rigor states (PSE, DFD, normal) are due to the difference in refractive index between myofibrils and sarcoplasm which is dependent on degree of myofibril shrinkage. In normal rigor and in the PSE condition, myofibrils shrink considerably which increases the refractive index and produces more light scattering. In contrast DFD muscle with higher pH also has less myofibrillar shrinkage hence the refractive indices of myofibrils and sarcoplasm are more nearly the same and light scattering is decreased.

Temperature. Temperature has a marked effect on color stability. If increased, oxyMb autoxidation rate is accelerated (George and Stratmann, 1952a; b; Brown and Mebine, 1969) because oxygen dissociates from Mb and autoxidation of deoxyMb occurs. Substantial differences in color were recorded both while the meat was stored in an oxygen-free gas atmosphere and during subsequent holding in air at different temperatures. Discoloration was more rapid with an increase in storage temperature from -1° C to +5° C (O'Keeffe and Hood, 1980a, b). Earlier, MacDougall and Taylor (1975) had also reported that even a small rise in temperature during display accelerated metMb formation; it was doubled by an increase of only 3° C. Unfortunately, typical running temperatures on commercial fan assisted convection display cabinets usually are above 4° C, the temperature

these workers regarded as nearly ideal for color stability

Snyder (1964) compared storage temperatures of 6, 2 and -2° C for fresh beef. The redness was greater at lower temperatures. Slower discoloration was attributed to decreased respiratory activity, when penetration of oxygen is deeper. Maximal oxidation of Mb to metMb occured closer to the meat surface with an increase in temperature due to decreased oxygen solubility (Brooks, 1929; Urbin and Wilson, 1958), increased oxygen utilizing systems in the meat (Urbin and Wilson, 1961; Snyder, 1964; Bendall, 1972) and occurence of maximal pO_2 for Mb oxidation (Ledward, 1970).

Mb oxidation rates are higher at a certain range of temperatures below the freezing point. Brown and Dolev (1963) observed a sharp increase at -10° C, a temperature at which the oxyMb solution (gel) appeared frozen. Freeze concentration effects on muscle components, catalytic effects of the ice crystals or favorable orientation of reactants in the partially frozen state with probable adjustment of the physical proximity of the Mb and oxygen molecules to a distance more favorable for oxidation reactions to occur, are factors that help to explain the unfavorable conditions at the temperature around -10° C (Fennema, 1973).

Zachariah and Satterlee (1973) studied the stability of porcine, ovine and bovine oxyMb in the range of -5 to -28° C. The pigments were least stable at -11 to -12° C, temperatures not usually encountered in storage but occuring during case defrost and causing deterioration of meat surface appearance. Sandberg (1970) compared frozen display temperatures of -12.2, -20.6 and -28.9° C, and noted the higher temperature resulted in darker color for both muscles studied (longissimus and psoas). Psoas major is less stable and its shelf life can be increased the most if stored at lowest temperature.

Light is another major factor that affects the oxidation rate of pigments. Kropf and Hunt (1984) presented an extensive review of the effects of display conditions on color of meat. Mention has also been made of the relation of higher temperature to aerobic microbial growth which contributes to color deterioration by reducing the oxygen tension on the meat surface (Robach and Costilow, 1962). Butler et al. (1953) demonstrated that bacteria in their logarithmic growth phase, when the oxygen demand is highest, cause discoloration of meat. In vacuum packaged meat, lactobacilli are the predominant bacteria (Ingram, 1962; Ordal, 1962; Pierson et al., 1970); they went from $10^3/cm^2$ to $10^8/cm^2$ in 15 days storage and represented 90% of the microflora (Pierson et al., 1970).

Packaging. Packaging has become an important factor in meat preservation; contamination after packaging is reduced and weight loss through evaporation is prevented. Early studies (Brooks, 1938) reported the effect of relative humidity on meat color deterioration. Dehydrated meat surfaces became dark and dull in appearance due to increased heme concentration and structural change, but hemoglobin apparently oxidized more slowly. Kraft and Ayres (1954) made a distinction between discoloration due to oxidation of pigments and that due to loss of water from the tissues. On the other hand, Ledward (1970) found that decreased water content of semitendinosus muscle led to increased metMb formation, but only at degrees of dehydration that led to marked discoloration due to causes like heme concentration and salt concentration which facilitate the oxidation process.

A major consideration in meat packaging is the modificaton of the gaseous atmosphere surrounding the meat. The gases primarily involved are O_2 and CO_2 in differing proportions. The shelf life of meat, as shown by color stability and

the extent and type of microbiological spoilage during storage, is influenced by the composition of the gaseous atmosphere in the package (Taylor, 1982).

Oxygen availability is probably the most important single factor to prevent Mb autoxidation. Meat must be maintained either at very low or at high oxygen tensions. Under refrigeration the respiration process is still active, consuming oxygen. If there is no exposure to air, e.g., vacuum package, the effective pO_2 is nearly zero. Residual oxygen will be depleted by enzyme activity, initially at a faster rate and thereafter at a slower rate over many days, while CO_2 is formed. Within 2 or 3 days, less than 0.5% O_2 and more than 20% CO_2 will appear in the vacuum package (Taylor, 1982) and most or all the ferrous Mb will ultimately be in the deoxy form. Initially metMb is formed from oxyMb; after a few hours metMb is reduced to deoxyMb. According to Pierson et al. (1970), the rate of reduction is decreased if the time of aerobic exposure between fabrication and packaging is increased. Therefore, autoxidation is lessened by reducing or eliminating oxyMb. Most oxygen should be depleted before reduction of metMb occurs (Watts et al., 1966). Upon exposure to air, the reduced pigment oxygenates rapidly again (Landrock and Wallace, 1965), but not so if the package is opened while metMb is the predominant pigment (Cutaia and Ordal, 1964).

If meat is held under a high oxygen atmosphere, the concentration of deoxyMb is kept low and redness is maintained because the surface layer of oxyMb is thicker and masks the layer of brown metMb formed at the limiting depth of O_2 penetration (usually about 4 to 5 mm). This delays the migration of metMb to the surface (Taylor and MacDougall, 1973). Underneath this layer, unchanged deoxyMb persists. This occurs within 1 to 3 days with high oxygen permeable wraps, such as PVC , depending on temperature. In retailing, the stability of meat color is relatively short with this type of film. Pierson et al. (1970) reported that beef

packaged in oxygen permeable film (MSAT 80 cellophane) at 0.5° C was unacceptable after 4 days of storage. When packaged in oxygen impermeable Capran 77K film, there was little difference compared with fresh meat for 10 days. Pirko and Ayres (1957) concluded that in films with low rates of gas transmission, metMb is converted to deoxyMb because the reducing activity of muscle is sufficient to keep pigments in the reduced form. These authors also found that films which resulted in maximum metMb formation on the sixth day of storage were those of highest oxygen permeability (cellophane MSAT 80, polyethylene 0.0015 in. and 80 FM 1 pliofilm).

Rikert et al. (1957) obtained better meat color stability in vacuum packages. Samples flushed with nitrogen or carbon dioxide had better color, attributed to residual oxygen removal. Nitrogen, as an inert gas, is ideal for gas packaging. If added to the package after evacuation, thus reducing the pressure on meat, it resulted in less exudate of retail cuts (O'Keefe and Hood, 1980b). According to Seideman et al. (1979a) there was no advantage in substituting 100% nitrogen environment for vacuum, except for less drip loss. Nitrogen does not affect meat color or inhibit bacteria.

Gas mixtures containing CO_2 (with nitrogen or air) are effective in extending storage life, more so if it is in high concentration. 100% CO_2 has also been used, but less frequently (Kraft and Ayres, 1952; Partman et al. 1970; Ledward, 1970; O'Keeffe et al. 1975; Ordonez and Ledward, 1977; Seideman et al. 1979a,b,c; 1980). Longer storage was achieved with higher concentration of CO_2, although in general the storage life was similar to that with vacuum packaging. In these systems oxyMb formation is not possible and metMb is favored. According to Lopez-Lorenzo et al. (1980), the longer the storage in CO_2/O_2 the higher the rate of Mb oxidation when the samples are subsequently exposed to air. Therefore, their

work applied only to wholesale cut storage. For beef (Clark and Lentz, 1973; Taylor and MacDougall, 1973; MacDougall and Taylor, 1975) and pork slices (Ordonez and Ledward, 1977) oxygen depressed Mb oxidation.

High pO_2 was reported not to increase benefit in color (Rikert et al., 1957). Zimmerman and Snyder (1969) used these conditions to inhibit respiration and prevent metMb formation. Beef slices were oxygenated at 5 atm O_2 for 12 hr and then wrapped in impermeable film. MetMb was formed on the surface because respiration seemed to proceed, depleting oxygen on the meat surface. But samples kept under high pO_2 for 12 days were still oxygenated, although deterioration (lipid oxidation and microbiological spoilage) had occured.

Studies on a modified atmosphere packaging system, using head space enrichment to about 90% O_2, resulted in prolonged acceptable color of meat slices as compared with those stored in air (Daun et al., 1971). Carbon dioxide is used to suppress bacterial growth when the holding times are prolonged (Clark and Lentz, 1973; Lopez-Lorenzo et al., 1980). CO_2 does not affect the rate of metMb formation (Ledward, 1970; Ordonez and Ledward, 1977). The volume of gas mixture relative to meat affects the concentration of gases during storage because the gaseous environment will be modified by gases diffused and respired from the meat. Taylor and MacDougall (1973) followed changes in gas volume and composition of beef stored in impermeable containers. The initial concentration of 80% O_2 was depleted to approximately 65% within 2 days, where headspace-to-meat ratio was 1 to 5. CO_2 level only changed slightly.

A mixture of 85% O_2 and 15% CO_2 increased color and shelf life by up to 10 days at 5° C compared with air (Clark and Lentz, 1973). At 1° C, time at 50% metMb formation was extended to about 13 days (Lopez-Lorenzo et al., 1980). Several other workers have concluded that mixtures of 75% to 85% O_2 with 25% to

15% CO_2 are most desirable (O'Keeffe et al., 1975; Ordonez and Ledward, 1977; Seideman et al., 1979b). Ordonez and Ledward (1970) reported off-colors and rancidity as negative effects of these environments, but 45 to 50% metMb is reached before TBA value of 5 is achieved (Lopez-Lorenzo, 1980), which suggests that rancidity is more critical during longer storage. Color, however, is a more limiting factor for retail shelf life.

Grinding. When meat is ground, flaked, thinly sliced or chunked the surface area is increased and more oxygen can penetrate it relative to the meat mass, which contributes to greater color instability (Hunt and Kropf, 1986).

Color problems have been noted in restructured beef steaks. Huffman and Cordray (1979) attributed discoloration both in the raw and cooked state to the addition of salt and other processing effects. Huffman (1980) noted that color contrast between chunked and flaked portions of steaks was greater when hot boned meat was used. MetMb formation and reduction in ground beef anaerobically packaged is affected by storage temperature. Because of the small particles, O_2 diffusion is very high and in the vacuum package the initial pO_2 allows autoxidation. Metabolic activity proceeds at a greater rate than in an intact muscle. This activity enhances the potential to reduce metMb, and is accelerated by temperature increases (Cutaia and Ordal, 1964). Autoxidation rates seem to be affected less markedly (Cutaia and Ordal, 1964; Brown and Dolev, 1963).

Ledward et al. (1977) compared the oxidation of ground and intact slices of beef during storage at 1° C. For intact muscles the rate of metMb formation varied with sample shape, thin slices being more color stable. The ground meat rates were similar for all muscles studied, although pH dependent. Grinding diminishes the reducing system. When the reducing system in intact muscle is depleted, the rate

of metMb formation is very rapid. Natural catalysts present in beef markedly accelerate metMb formation at the aerobic surfaces and the natural reducing systems present fail to maintain the equilibrium level after a certain time.

Aging. O'Keeffe and Hood (1980a; b) have shown fundamental effects of age post mortem and storage temperature on the color stability of beef. The influence of O_2 consumption rate on the depth of oxyMb layer was also reported. Meat aged for only 3 days has a more unstable color than meat aged for 7 days. It has higher O_2 consumption rate compared to 7 days conditioning, which prevents the formation of a deep layer of oxyMb and, therefore, discolors more rapidly.

However, MacDougall and Rhodes (1972) suggested that both the better oxygenation and the faster accumulation of metMb of conditioned meat result from a decrease of the enzymatic activity. A thicker layer of oxyMb forms in conditioned meat. As the rate of O_2 consumption diminishes by the depletion of the glycolytic cycle, O_2 can penetrate faster and deeper into the tissue resulting in a thicker layer of oxyMb. The metMb formed at the O_2 penetration limit is not re-reduced to deoxyMb because such intermediates as NADH are no longer formed.

Muscle discoloration rate increased with postmortem aging. Gluteus medius and vastus lateralis muscles were affected most. They produced about 0.5% more metMb after 7½ hr display at 25° C for each day's aging postmortem. This effect is also apparent at lower temperatures. After 96 hr storage at 5° C, gluteus medius was similar in discoloration to that after 7½ hr at 25° C, ie., approximately 0.5% additional metMb per day. Postmortem aging and increased penetration of O_2 is related to decay in mitochondrial activity. It also accounts for the increased variability which occurs within a particular type of muscle (Hood, 1980).

Meat is normally aged to improve tenderness and eating quality (Joseph, 1971). In the case of pre-packaged ground beef, centralized processing may result in lower cost and extended shelf life but product has more age at purchase time. The bloomed color of conditioned meat is superior because increased O_2 penetration results in a deeper layer of oxyMb, but has a poorer color stability, because oxyMb is not maintained during subsequent pre-packaging and display (MacDougall, 1972, cited in Hood, 1980).

Meat aged for 3 to 4 weeks has a more rapid discoloration when exposed to air than meat aged for 1 week, and is dependent on muscle type if held in anaerobic environment. Decrease in shelf life was 41, 32 and 35% for psoas major, gluteus medius and semimembranosus muscles, respectively, after 4 weeks compared with 1 week of aging. When aged for less than a week, greater instability was reported for both stable longissimus dorsi or less stable biceps femoris, compared to longer aging. Age has a combined effect with temperature (O'Keeffe and Hood, 1980b). As pH decreases, the reducing system is affected and the rate of metMb reduction decreases (Cutaia and Ordal, 1964).

Pre- and postrigor deboning. The effect of muscle sample depth from the surface in a carcass on the rate of postmortem glycolysis was reported by Tarrant and Mothershill (1977). A darker muscle color was noted in muscles excised at 1 or 2 hr post mortem then those excised at either 4 or 48 hr. Rigor state has also been shown to affect color of the finished restructured steak.

Huffman (1980) found the color contrast between chunked and flaked portions of steaks was greater when hot boned meat was used. Restructured steaks made from prerigor beef were less desirable in color than steaks from postrigor beef.

The rate of chill has a marked influence on postmortem changes in muscle. Muscles located nearer the surface or with lesser transverse surface area chill at a faster rate than those located deeper and a gradient of rate of temperature decline occurs between these two. Deeper in the carcass, the temperature drops slower and the enzymatic activity remains higher but is depleted more rapidly. Differing pH decline between and within muscles affects muscle properties such as water binding capacity. Light scattering is higher at low pH values and the muscle appears lighter (Huffman, 1980).

Color stability is also affected. MetMb formation was lower in prerigor, compared to postrigor ground pork stored under aerobic conditions (Judge and Aberle, 1980). At higher pH values pigment oxidation is less favored and the reducing enzyme activity is favored.

Prerigor meat generally is darker and may not oxygenate to the same bright red color as postrigor meat. A faster pH decline of electrically stimulated meat favors pigment oxygenation, i.e., the increase in oxyMb content (Tang and Henrickson, 1980; Savell et al., 1978).

The actively respiring mitochondria in prerigor meat prevent extensive oxygenation of meat exposed to air. If rotenone, a mitochondrial inhibitor, is added, the meat turns bright red. A similar effect was obtained treating prerigor samples with pH 5.3 buffer (Cornforth and Egbert, 1985).

Dark cutting beef is associated with a high ultimate pH and reduced O_2 diffusion (Lawrie, 1958). Ashmore et al. (1971; 1972; 1973) studied the functional capacity of mitochondria isolated from dark cutting muscle and found that they remain active due to higher pH. However, a decrease in activity occured with time after death, as compared to meat with normal pH drop , where the rate of mitochondrial activity loss was higher. They concluded that the color is a cut

surface phenomena, where the relatively high oxygen consumption rate maintains the Mb in the deoxy form.

Metmyoglobin reducing systems. The accumulation of metMb at the surface of fresh beef depends on two opposing mechanisms: (a) the autoxidation of the reduced pigment in the presence of oxygen (George and Stratman, 1952a; b) and (b) the enzymatic reduction of metMb to deoxyMb (Stewart et al., 1965a; Ledward, 1970, 1972). The in situ metMb reduction has been studied more from an empirical approach and the mechanism of reduction in meat and exogenous influencing factors are not yet well understood (Giddings, 1974).

Dean and Ball (1960) did some early analytical work on the reduction of surface metMb in vacuum packaged fresh beef cuts and attributed it to "natural processes". Cutaia and Ordal (1964) assessed oxidation/reduction of Mb at the surface of fresh beef cuts by means of reflectance spectrophotometry and found that meat pH and fat content were major factors influencing autoxidation of Mb. Later the metMb reducing activity (MRA) was studied on induced metMb, either oxidizing Mb with ferricyanide, $K_3Fe(CN)_6$ (Stewart et al., 1965a; b) or in low partial pressure (1% O_2) atmosphere (Ledward, 1970; 1972; Ledward and MacFarlane, 1971). The reduction of metMb in either aerobic or anaerobic conditions, was followed by reflectance spectrophotometry . Reflectance readings were converted to K/S ratios. Ratios at 572 and 525 nm (metMb) and at 474 and 525 nm (deoxyMb) were obtained. Enzymatic reduction at the surface of ground beef samples stored 10 to 13 days at 1° C was studied after samples were treated with ferricyanide solution and the percentage of total pigment reduced one hour later in anoxic conditions at room temperature (22° C) was determined (Stewart et al., 1965a; b). Reduction ranged from 20 to 100%. Ledward (1970) used thin (2 mm)

beef slices which were held for 24 hr in 1% O_2 atmosphere. The percentage of metMb formed was determined then and again after an additional 24 hr in air. The percentage of metMb reduced during the 24 hr was calculated and plotted vs. the equilibrium metMb concentration. In this experiment the correlation coefficient between the equilibrium metMb concentration on beef slices stored 7 days in air and the aerobic MRA (reduction rate from 10 to 25%) was highly significant (r=-0.94) but not to anaerobic MRA as determined by Stewart et al. (1965a). This suggests two different mechanisms of reduction. Lanier et al., (1978) studied the reduction of metMb in ground beef, beef slurries and extracts held in air, nitrogen or carbon monoxide (CO) air mixtures. Significant metMb reduction occured in aerobic extracts but not in ground beef or slurries in the presence of ferricyanide. A nonenzymatic electron transfer was demonstrated in extracts under these conditions. CO accelerated metMb reduction, even in the presence of air, with the rate of reduction dependent upon CO concentration. Possible mechanisms for this enhanced reduction were explored.

Discoloration first becomes apparent at the cut surfaces. With ground meat, discoloration also takes place in the interior because of higher surface exposure to oxygen. An anoxic environment develops rapidly as oxygen is depleted, causing reduction of the pigment (Giddings, 1974).

In vacuum packages, the residual O_2 causes metMb formation in a few hours because the rate of oxidation is greater than enzymatic reduction at low O_2 tension (Rikert et al., 1957). After most O_2 has been converted to CO_2, the reducing activity of the muscle prevails (Taylor and MacDougall, 1973) and will keep the pigment in a reduced state throughout storage.

MetMb reduction in vivo has been studied in association with an anemic disorder, methemoglobinemia, that results from insufficiency or absence of the

reductase enzyme in the erythrocytes. Cytochrome b5 is the physiological intermediate. Such a system has not been demonstrated in mammalian muscle fibers (Giddings, 1974).

A fraction of the myoglogin in the living cell becomes oxidized at the O_2 tension in the interior of the muscle fiber and it is expected that a "reductase" system exists (Giddings, 1974). Such systems have been studied in bluefin tuna and mackerel (Al-Shaibani et al, 1977) and dolphin (Shimizu and Matsuura, 1971). But an artificial mediator, methylene blue, was used in the assay to which there is no obvious physiological counterpart.

Hagler et al. (1979) purified the first metMb reductase from beef heart that does not use mediators. This and similar enzymes purified from blue white dolphin muscle use ferrocyanide or cytochrome b and NADH for reduction of metMb (Matsui et al., 1975, cited in Livingston and Brown,1981).

Stewart et al. (1965a) reported that MRA varied among beef ribeye muscles from different animals and increased with increasing pH (5.1 to 7.1) and temperature (3 to 35° C). During refrigerated storage, MRA of intact cuts declined slightly but that of ground beef declined more rapidly. They proposed a mechanism dependent on the availability of NAD concentrations and oxygen uptake. MRA and NAD concentrations were highly correlated in ground lamb and beef, but not in pork.

In situ levels of NADH and other possible non enzymatic reductants are important factors in regulating rates of metMb reduction. Low O_2 tension, which does not inhibit enzymatic metMb reduction directly, may serve to increase the in situ NADH levels (Livingston and Brown, 1981).

Watts et al. (1966) suggested that the latter may be generated via lactate dehydrogenase (LDH) which Giddings (1974) considered thermodynamically

unfavorable. They also reported that NADH is essential for metMb reduction and that both NADH and succinate are involved in accelerating the reduction by removal of tissue oxygen via mitochondrial electron transport (essentially anoxic conditions were observed to be required). The MRA rate was reported to be increased by treatment of raw ground beef with papain. Saleh and Watts (1968) found that MRA was increased by addition of substrates having pyridine nucleotide-linked dehydrogenases plus NAD or NADP, indicating that the substrate and/or pyridine nucleotide was limiting. The cofactors seemed to be more limiting since the additon of NAD or NADH alone accelerated MRA. Hall et al. (1972, cited by Giddings, 1974) found that the rate of reduction of pyridine nucleotides controls the rate of MetMb reduction. They also reported that the dehydrogenases remain potentially active in the muscle cell for significant periods postmortem. The findings that the rate of Mb autoxidation is greater than the rate of reduction at low O_2 pressures was also confirmed on beef slices by Ledward (1970). The high surface metMb content induced on samples in low O_2 atmosphere was reduced slowly when exposed to air, the rates depending upon temperature. MRA was maximal in anoxia. Low pH accelerated autoxidation and retarded MRA.

NADH loss is known to be accelerated with grinding (Newbold and Scopes, 1971), confirming the findings of Watts et al. (1966) that ground beef rapidly loses its ability to reduce metMb and the accumulation of metMb is more rapid than in sliced beef (Ledward and MacFarlane, 1971).

Accumulation of metMb varies markedly from muscle to muscle (Ledward, 1970; 1971) and these variations have been attributed to differences in the activity of the aerobic MRA naturally present in beef (Ledward, 1972).

According to Greene (1969), if the meat has adequate MRA, anoxic packaging can prevent metMb formation and lipid oxidation. If antioxidants are

added to fresh meat, both pigment and lipid oxidation can be retarded even in aerobic conditions. Free radicals from lipid oxidation were a major cause of protein denaturation but they seem to first oxidize the heme (Greene et al. 1971). Govindarajan and Hultin (1977) reported that the reactions involved in the early stages of lipid oxidation are important in the oxidation of the meat pigment. Therefore an assumption can be made that the more metabolically active a cut of fresh meat is, the greater the oxygen consumption by the tissues and the vulnerability of lipid and Mb to oxidation. Oxalate inhibited both lipid and Mb oxidation.

Even in color stable muscles such as the longissimus dorsi, the reducing system becomes depleted in time as compared to psoas major which fails to maintain the equilibrium needed to maintain color stability (Ledward et al. 1977). MacDougall (1982) reported that the lighter colored, more denaturated inner portion of the semimembranosus muscle of beef that was chilled more slowly had less MRA.

MRA patterns followed that of reduced O_2 demand with storage time. A lower O_2 uptake appears to play a more decisive role in delaying surface discoloration (Atkinson and Follett, 1973). Pierson et al (1970) reported that the rate at which the surface metMb of fresh beef underwent reduction upon vacuum packaging, ie., the rate and extent of reduction of any oxidized pigment in vacuum and also the rate and extent of subsequent oxygenation when exposed to air, decreased as time between slicing and vacuum packaging was increased.

Zimmerman and Snyder (1969) also reported that surface oxyMb was first autoxidized, followed by reduction, when oxygenated fresh beef cuts were wrapped in gas-impermeable film. Malonic acid, a succinic dehydrogenase inhibitor, retarded the reduction of metMb. Its effect on MRA was explained based on interference

with residual oxygen removal rather than pyridine nucleotide reduction. A combination of factors involved in the gradual loss of MRA in postrigor meat, especially when exposed to air, include fall in tissue pH, depletion and/or degradation of substrate and cofactors, oxidative/deteriorative changes and loss of cofactors and enzymic activities, including ultimate complete disintegration of mitochondrial particles along with impairment of key enzymatic activities (Giddings, 1974).

Keeping red meat anoxic, cool, clean and moving, are very important factors to be observed by the fresh red meat industry to minimize negative effects of oxygen on tissue components (Giddings, 1974).

Oxygen uptake and diffusion. In the living animal, the external oxygen required for metabolic activities is supplied by the circulatory system. With the numerous changes that meat undergoes, O_2 availability depends on the surrounding environment, Mb concentration and the remaining respiratory enzyme activity and contributes to the state of the Mb (Grant, 1955).

Enzyme degradation rates vary widely under the same storage conditions. Cheah (1971) showed that a large proportion of intact mitochondria are present in ox neck muscle after 6 days postmortem storage at 4° C. The succinoxidase system was found to be significantly more labile. Decreasing tissue pH was considered the key factor influencing postmortem mitochondrial deterioration. As indicated by Ashmore et al. (1973), when muscle pH remains high, whether due to excessive adrenalin injection or to natural causes (dark cutting meat), mitochondrial respiration remains high and Mb is in the deoxy state.

Oxygen uptake by the meat tissue and the rate of O_2 diffusion into the tissue is regulated by the physical condition at the meat surface (Lawrie, 1958)

and determines the O_2 tension in the tissue fluid. Temperature, ATP turnover rate and mitochondrial oxidation play an important role in the O_2 consumption rates in prerigor beef (Bendall, 1972).

Oxygen uptake is not restricted to the meat surface. Brooks (1929) demonstrated that the depth of O_2 penetration in muscle was determined by the rate of diffusion of O_2 into the tissue and the consumption of the gas by the tissue; they are inversely related. Discoloration was restricted to this relatively thin, superficial zone. The equilibrium depended on temperature, pO_2, time and muscle conditions. Increasing temperature tended to decrease O_2 penetration while higher pO_2 or longer exposure caused a deeper O_2 penetration. The O_2 penetration was rapid initially and then slowed down to a linear rate, attributed to a decrease in the tissue O_2 consumption. Morley (1971) reported similar results, but related with aerobic metabolism of contaminating microorganisms on the meat surface.

The rate of Mb oxidation increased with decreasing pressure and reached a maximum at low pressure of about 4 mm O_2 at 0° C). George and Stratman (1952a; b) found a maximum of Mb oxidation at a pO_2 of 1 mm Hg with half of the total ferrous Mb in the deoxy form, while Ledward (1970) found maximal formation of metMb in fresh beef at pO_2 from 6.0 to 7.5 mm Hg.

When meat is exposed to air, oxygen penetrates gradually into the muscle, determined by relative rates of diffusion and uptake. Three different color layers are usually formed. OxyMb layer, formed first on the surface, becomes deeper as O_2 diffuses inward. At a few mm below the surface, there is a region where the pO_2 is in the optimum range for metMb formation and a brown layer can be seen in this region. Still further below the surface, where pO_2 is lower, the potential for metMb falls, and reduced Mb predominates (Taylor, 1972). The depth of O_2 penetration is proportional to the square root of its partial pressure. With 80% O_2

at the surface, the oxygenated layer is twice as thick as in air and the metMb layer at the penetration limit is still far enough from the surface to remain obscured for several days (Taylor, 1982).

Oxygen consumption rate decreased with age of meat postmortem due to depletion of substrate and coenzymes and degradation of enzymes involved in mitochondrial respiration. Decreases in temperature and pH of meat cause decreased oxygen consumption rates (OCR) (Bendall, 1972; Urbin and Wilson, 1961). Atkinson and Follett (1973) reported that O_2 uptake and NAD concentrations of ground lamb and beef samples were highly correlated; for pork the correlation was lower. The O_2 consumption rate was inversely proportional to the rate of discoloration. Beef, more stable, had the lowest OCR and lamb, less stable, had higher OCR. MRA and OCR have, therefore, an inverse relationship, and a lower O_2 uptake appears to play a more decisive role in delaying surface discoloration. A relationship with retail display life was also found. Lamb muscle has a short display life due to very rapid Mb autoxidation on the meat surface when exposed to air. Its early postmortem O_2 uptake is very high and during maturation, the decline in O_2 demand appeared to be related to a corresponding decrease in NAD concentration. Addition of NAD or NADH caused an increase in O_2 utilization.

Patterns of O_2 uptake differ for different species. It decreases with maturation. Retail display life depends upon the oxygen demand of the tissue (Atkinson and Follett, 1973). Differences were observed in the OCR for different muscles of the same species (Bendall and Taylor, 1972). Zimmerman and Snyder (1969) studied the effects of respiratory inhibitors on metMb accumulation by using high O_2 pressures and oxidizing sulphydryl groups. MetMb could only be retarded by this treatment. Bendall and Taylor (1972), have shown a higher postrigor O_2

consumption rate for beef biceps femoris with relatively unstable color than for beef longissimus dorsi muscle.

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Chapter II

EFFECT OF OXYGEN EXPOSURE DURING PROCESSING ON GROUND BEEF COLOR STABILITY

Summary

Semimenbranosus muscle (48 hr postmortem) of 3 beef carcasses was trimmed, cut and ground in a nitrogen atmosphere, compared to 48 hr exposure of muscle to air before grinding. Samples stored in vacuum 7 and 14 days were displayed under natural fluorescent lighting at 3° C for 5 days and these color traits were measured: % metmyoglobin (metMb), difference of 630 and 580 nm reflectance (R_{630}-R_{580}), HunterLab values, saturation index, hue angle and visual score. MetMb increased faster ($p<0.05$) for samples processed in air after 14 days in vacuum, but not for those stored 7 days. Hunter L values of 14 day samples were higher ($p<0.05$) when processed in air; treatment did not affect Hunter a values. Samples stored 14 days in vacuum oxygenated better and were redder throughout display (higher R_{630}-R_{580}, lower visual score, higher Hunter L) than for 7 days storage. Samples processed in air, had very little oxyMb formation. Treatment did not affect ($p>0.05$) aerobic metmyoglobin reducing activity (MRA), although anaerobic MRA was greater for the nitrogen treatment

Introduction

The bright red color of ground beef is an important quality characteristic considered by the purchaser of fresh meat in the retail market. Much ground beef in the USA is distributed to the retail markets from fabrication operations as coarse ground and packaged in oxygen impermeable casing. In retail stores the meat is fine ground and repackaged in an oxygen permeable film.

The development and stability of the bright red color during display depends on a number of factors that have to be controlled during processing and display. Failure to achieve the bright red color has been reported in the industry and represents product loss.

Partial oxygen pressure (pO_2) is the major factor, besides temperature and pH, that affects the chemical state of myoglobin (George and Stratman, 1952a; b). At high pO_2, oxygenation occurs spontaneously and oxymyoglobin (oxyMb) predominates (Govindarajan, 1973). Low storage temperature inhibits deoxygenation of oxyMb by suppressing respiratory enzyme activity (Lawrie, 1974) and favors oxygen penetration into the tissue (Snyder, 1964). Higher temperatures accelerate any remaining respiratory activity (MacDougall and Taylor, 1975; Bendall, 1972), depleting reducing activity and favoring pigment oxidation (discoloration). Conditions that favor low oxygen uptake have an important role in delaying surface metMb accumulation. In practical terms, low temperature and minimal exposure to air during processing and before packaging in anoxic conditions should be beneficial (Pierson et al., 1970).

Enzymatic metmyoglobin reducing activity (MRA) is another factor influencing meat surface discoloration (Stewart et al., 1965; Ledward, 1970; 1972). MRA retention is related to chill rate as muscles chilled slowly have less MRA

(MacDougall, 1982). MRA is depleted with increasing time postmortem (Atkinson and Follet, 1973) due to depletion and enzyme degradation (Urbin and Wilson, 1961; Bendall, 1972). The grinding process affects MRA, depleting it faster. The result is less stable color and faster discoloration as compared to intact meat slices (Ledward, 1977; Stewart et al., 1965).

The purpose of this study was to verify if ground beef of better color stability could be obtained by reducing the exposure of meat to air during processing.

Materials and Methods

Semimembranosus muscles were excised at 48 hr postmortem from one side of three chilled beef carcasses from approximately 2 year old bulls, with ultimate pH of 5.41 to 5.49. The muscles were placed in vacuum bags to minimize oxygen diffusion into the muscle and immediately transferred to a biological hood, kept at 2 to 4° C. Air was withdrawn from the hood which was then maintained with a nitrogen atmosphere by flushing with nitrogen several times so that the oxygen content was not higher than 0.15%, as analyzed by the MOCON - Oxygen Analyzer LC 7007. The following processing steps were conducted under nitrogen. Excess fat and about 2.5 cm of the meat surface were trimmed and discarded. The muscle was cut into approximately 5 cm cubes which were alternately assigned to one of two treatments to even out the effect of differential chilling rate and muscle morphology.

Half of the samples were assigned to the nitrogen treatment and mixed and coarse ground using an Oster grinder, mixed again and 80 to 100 g samples were taken randomly, formed into patties on styrofoam trays, wrapped in

polyvinylchloride (PVC) film, placed individually in vacuum bags which were immediately vacuum sealed after removal from the hood. These packages were stored in the dark at 3 to 4° C for either 7 or 14 days.

The control group of samples were removed from the hood (cut in cubes), stored in a thin layer covered with polyvinilchloride (PVC) film in the dark at 3 to 4° C for additional 48 h. All other conditions were similar for both groups.

Metmyoglobin Reducing Activity

MRA was determined by the aerobic method using a modification from Ledward (1972) as described by Sleper et al., 1983). After 14 days storage, six PVC wrapped patties of each treatment were removed from the vacuum packages, excess moisture was wiped from the surface and the patties were placed in an anaerobic incubator in a 1% O_2 environment for 48 hr at 3 to 4° C to induce metMb formation. The incubator was evacuated and flushed several times with a gas mixture of 1% oxygen and 99% nitrogen. Gas samples from the incubator were monitored for oxygen level with the MOCON instrument. Each sample was removed from the incubator while it was being continuously flushed with the gas mixture to maintain proper atmosphere in the incubator. Samples were scanned spectrophotometrically at three different locations for percentage reflectance at 525 and 572 nm with a HunterLab D-54 spectrophotometer. MetMb reduction was measured by calculating % metMb at 0, 2, 4, 6, 8, 10, 12 and 24 hr air exposure at 3 to 5° C.

The MRA also was determined by the anaerobic method (Stewart et al., 1965; Sleper et al., (1983). After 14 days storage, six patties of each treatment were removed from packages and metMb formation was induced chemically with an excess of 1% potassium ferricyanide solution [$K_3Fe(CN)_6$] applied on the sample

surface for 1 min. Excess solution was blotted, and samples were allowed to oxidize in air for 30 min at room temperature before repackaging in vacuum. The reduction of metMb under anaerobic conditions was measured at three locations on the samples immediately after packaging (time 0) and after 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min by reflectance spectrophotometry at 525 and 572 nm and calculating % metMb.

Pigment Oxygenation

Three vacuum packaged patties of each treatment that had been stored for 14 days, were used to evaluate the rate of oxygenation when exposed to air. Percentage reflectance at 580 and 630 nm and Hunter L, a and b values (Hunter, 1958) with Illuminant A were measured immediately after opening the package and after 1, 2, 3, 4, 5, 8, 15, 30 and 60 min. Samples were scanned at three different locations through the PVC film and remained at room temperature during evaluation.

The difference of reflectance at 580 and 630 nm (R_{630}-R_{580}), which is linearly related to the relative proportions of the two pigment forms metMb and oxyMb on the meat surface, was used as indicator of redness during oxygenation.

Display Color Stability

Semimembranosus muscles from three additional animals (2 steers and 1 heifer) were prepared as described above. Meat cubes assigned to both treatments were first coarse ground in an Oster grinder, vacuum packaged and stored at 3° C. After 7 and 14 days of storage, the meat was reground through a fine plate, 3.2 mm plate, formed into patties, placed on styrofoam and wrapped with PVC film. Five samples of each treatment were allowed to oxygenate one hour at 4° C.

Reflectance measurements at 525, 572, 580 and 630 nm and Hunter L, a and b values with Illuminant A were taken at three different locations on each sample with a Hunterlab D-54 reflectance spectrophotometer and visual scores were taken. Display study was conducted in an open top case under 970 lux Natural fluorescent lighting for 24 hr per day at 2 to 4° C for 5 days. Visual color evaluation was done by a trained 5 member panel who evaluated overall lean color using the KSU beef color scale (Kropf et al., 1971) to the nearest 0.5 point (1 = very bright red, 2 = bright red, 3 = slightly dark red or brown, 4 = dark red or brown, 5 = extremely dark red or brown). Sample scoring, both visually and spectrophotometrically, was done at 0 (before display), 1, 3 and 5 days of display.

Calculation of MetMb

Calculation of metMb was based on transforming the reflectance values to K/S ratios using Kubelka-Munk's equation ($K/S = (1 - R)^2 / 2R$), which takes into account the changes in the light flux incident on the sample, expressed by the absorption and scattering coefficients, respectively (Francis and Clydesdale, 1975). Ratios of K/S values at 572/525 nm were calculated to determine percentage metMb. The 572/525 nm ratio is minimal with 100% metMb (oxidation with 1% $K_3Fe(CN)_6$ solution) and maximal when only deoxyMb or oxyMb are present, and is determined experimentally. The ratios we used were 1.40 and 0.56 for 0% metMb and 100% metMb, respectively (Stewart et al., 1965) and the equation used follows:

$$\% \text{ metMb} = \frac{K/S\ 572/525\ (0\%\ \text{metMb}) - K/S\ 572/525\ (\text{sample})}{K/S\ 572/525\ (0\%\ \text{metMb}) - K/S\ 572/525\ (100\%\ \text{metMb})} \times 100$$

Differences between percentage reflectance at 630 and 580 nm were used to indicate muscle redness, larger values being associated with higher oxyMb (brighter red).

Hunter a and b data were reduced to hue angle, $\tan^{-1}$ b/a, and saturation index, $(a^2+b^2)^{\frac{1}{2}}$. These values along with Hunter L values were suggested as color attributes to specify a color using the HunterLab system (Setser, 1984).

Statistical Analysis

Data were analysed by analysis of variance procedures for a multi split-plot design and means were compared by LSD test (SAS Institute, 1979; Cochran and Cox, 1957)

Results and Discussion

Display Color Stabilility

Table 1 summarizes the results of an analysis of variance of the display study. A three way treatment, vacuum storage time and display time interaction (TRT*ST*T; Table 1) was calculated for %metMb ($p<0.01$) and Hunter a value ($p<0.05$) measurements. MetMb increased faster on samples processed in air than in nitrogen when stored 14 days, but not for samples previously stored in vacuum 7 days. Samples stored for 14 days had greater metMb accumulation at day 3 and 5, when processed in air (Table 2; Fig. 1).

Hunter a values declined from day 0 to 1, and then remained fairly constant. The samples stored 7 days in vacuum did not show the lowest Hunter a values, although they had highest % metMb. Samples stored 14 days had no significant treatment effect ($p>0.05$) (Table 2; Fig. 1).

An interaction ($p<0.05$) of treatment and storage time (TRT*ST) was calculated for Hunter L and b and saturation index (Table 1). Hunter L values at 14 days (Table 3) were higher for the nitrogen treatment along with lowest % metMb shown in Table 2, compared to air with 14 days storage. A treatment effect was not shown for the 7 days vacuum storage.

The effect of 7 and 14 days vacuum storage x display time (ST*T) was significant for all parameters measured (Table 4; Fig. 2). Samples stored 14 days oxygenated better and were redder throughout the display as indicated by higher $R_{630} - R_{580}$ values, lower visual scores up to the 3rd day and higher Hunter L values. The latter decreased over time. The higher saturation index for 14 day storage indicates purer red color, ie., less brown metMb pigment. Hue angle increased for the samples stored 14 days. A higher hue angle should indicate a less red, more yellow color. This measurement should not be affected by heme pigment concentration.

Pigment Oxygenation

Table 5 contains a summary of the significance levels of the effects of processing ground beef in air versus nitrogen on rate of oxygenation and aerobic and anaerobic MRA.

There was a treatment x time (TRT*T) interaction ($p<0.05$) on sample redness ($R_{630} - R_{580}$). Samples processed under nitrogen oxygenated better as shown by a higher number and the value tended to increase in the first 30 minutes (Table 6). The air treatment samples had very little change with time.

A significant time effect ($p<0.01$) on Hunter L and a values as well as on saturation index and hue angle was found. Hunter L increased during oxygenation, but Hunter a declined, an unexpected result. Hunter b values fluctuated and did

not follow a pattern. Saturation index and hue angle decreased with time. Saturation index is $(a^2 + b^2)^{\frac{1}{2}}$; since b did not change much, it is influenced mostly by the decrease in a. The decreasing hue angle suggests a purer red color with more time.

Aerobic and Anaerobic MRA

Aerobic MRA determination resulted in metMb levels that were not different ($p>0.05$) for samples processed in nitrogen compared with those in air. The initial mean metMb concentration of 78.7% was reduced to 54.8% after 24 hr (Table 7). The metMb concentration of the anaerobic MRA determination declined from 78.1% to 27.1% (Table 8).

A treatment x time (TRT*T) interaction for anaerobic MRA was noted ($p=0.054$). Samples processed under nitrogen initially had lower metMb levels compared to the air exposed samples (Fig. 4) up through 5 min. Then the concentration of metMb remained practically constant through 24 hr. This suggests at least a short time advantage in MRA for samples trimmed, cut and ground in nitrogen. We are not recommending processing under nitrogen, but these results support the importance of minimizing air exposure of beef trim, an idea clearly shown by Pierson et al. (1970).

When meat is vacuum packaged it usually has a layer of oxyMb on the surface, a thin layer of metMb and most of the pigment in the deoxy form. In the anoxic package, the residual oxygen is used up with time and a layer of metMb is formed on the surface for several hours, before all pigment is reduced to deoxyMb by the enzymatic reducing system. The discoloration pattern of ground beef is not uniform because of variation of pO_2 in the meat and the destructive effect of grinding on the reducing system. This spotty aspect and the fat particles may

cause variability in the measurements used in this study.

Meat tissue rapidly becomes depleted of oxygen postmortem. Oxygen from the air diffuses into the tissue surface and deeper. A certain amount is continuously being utilized by the respiratory enzymes. This utilization is potentiallly directly related to the temperature, so that oxygen available is constantly decreasing (Urbin and Wilson, 1961), more so at higher temperatures. If meat is held at temperatures close to 0° C and the exposure to air is limited before vacuum packaging, more enzymatic reducing activity is retained and will keep the meat from metMb development for a longer time during display in aerobic conditions. This was observed in some of the color traits measured under display and by anaerobic MRA determination suggesting preservation of a more active reducing system for samples with less exposure to oxygen during processing.

Loss of MRA though seems to be consequence of both grinding and incorporation of oxygen, since controlling exposure to air resulted in some advantages on color stability. Our data tend to suggest that grinding beef causes a loss of MRA that is independent of the incorporation of oxygen that occurs in grinding.

Table 1 - Significance of display color effects of ground beef in air and nitrogen after 7 and 14 days vacuum storage.

Color trait	TRT	ST	T	TRT*ST	TRT*T	ST*T	TRT*ST*T
MetMb (%)	**	**	**	n.s.	n.s.	**	**
$R_{630}-R_{580}$	n.s.	**	**	n.s.	n.s.	**	n.s.
Visual score	* (.057)	*	**	n.s.	n.s.	**	n.s.
Hunter L	*	**	**	**	n.s.	**	n.s.
Hunter a	n.s.	*	**	*	n.s.	**	*
Hunter b	n.s.	**	**	*	n.s.	**	n.s.
Saturation index	n.s.	**	**	*	n.s.	**	n.s.
Hue angle	n.s.	**	**	n.s.	n.s.	**	n.s.

**P<0.01
* P<0.05
n.s. P>0.05
TRT = treatment ST = vacuum storage time T = time

Table 2 - Effect of processing ground beef in air and nitrogen on MetMb formation and Hunter a values during display at 3°C.

Color trait	Vacuum storage (days)	Treatment: Air, Display time (days) 0	1	3	5	Treatment: Nitrogen, Display time (days) 0	1	3	5
MetMb (%)	7	$7.6^{a,m,x}$	$8.7^{a,m,x}$	$41.9^{b,n,x}$	$54.0^{c,m,x}$	$0.7^{a,m,x}$	$7.1^{b,m,x}$	$41.0^{c,n,x}$	$53.1^{d,n,x}$
	14	$0.5^{a,m,x}$	$0.5^{a,m,x}$	$28.3^{b,m,y}$	$51.1^{c,m,y}$	$0.5^{a,m,x}$	$0.5^{a,m,x}$	$15.1^{b,m,x}$	$43.6^{c,m,x}$
Hunter a	7	$40.1^{c,n,x}$	$31.7^{a,m,x}$	$32.6^{a,m,x}$	$34.4^{b,n,y}$	$38.7^{c,m,x}$	$32.9^{ab,m,y}$	$32.7^{a,m,x}$	$33.9^{b,n,x}$
	14	$37.8^{b,m,x}$	$33.0^{a,n,x}$	$32.1^{a,m,x}$	$32.4^{a,m,x}$	$39.3^{c,m,y}$	$33.6^{b,n,y}$	$32.4^{a,m,x}$	$32.7^{ab,m,x}$

^{abcd}Means within a treatment and storage time with the same superscript letter are not different ($P>0.05$) for display time effects.

^{mn}Means within a treatment and day of display with the same superscript are not different ($P>0.05$) for storage time effects.

^{xy}Means within a day of display and storage time with the same superscript are not different ($P<0.05$) for treatment effects.

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Table 3 - Effect of processing ground beef in air and nitrogen on Hunter L and b values and saturation index for samples displayed after 7 and 14 days vacuum storage.

Color trait	Vacuum storage (days)	Treatment	
		Air	Nitrogen
Hunter L	7	$10.8^{a,x}$	$11.0^{a,x}$
	14	$13.6^{a,y}$	$15.6^{b,y}$
Hunter b	7	$25.8^{a,x}$	$26.1^{a,x}$
	14	$30.9^{a,y}$	$32.8^{a,y}$
Saturation index	7	$32.3^{a,x}$	$32.4^{a,x}$
	14	$39.2^{a,y}$	$41.4^{a,y}$

^{ab}Means in the same row with same superscript are not different (P>0.05).

^{xy}Means within treatment with same superscript are not different (P>0.05).

Table 4 - Color of ground beef, stored 7 and 14 days in vacuum, during display at 3°C.

Color trait	7 days, Display time (days)				14 days, Display time (days)			
	0	1	3	5	0	1	3	5
$R_{630}-R_{580}$	$16.5^{a,x}$	$22.7^{c,x}$	$20.1^{b,x}$	$17.4^{a,x}$	$26.9^{c,y}$	$27.0^{c,y}$	$23.4^{b,y}$	$21.4^{a,y}$
Visual score	$1.7^{a,y}$	$2.2^{a,y}$	$3.5^{b,y}$	$3.7^{b,x}$	$1.3^{a,x}$	$1.9^{a,x}$	$3.2^{b,x}$	$3.9^{b,x}$
Hunter L	$16.9^{d,x}$	$11.3^{c,x}$	$8.5^{b,x}$	$6.9^{a,x}$	$24.2^{d,y}$	$15.3^{c,y}$	$10.7^{b,y}$	$8.1^{a,y}$
Hunter b	$28.7^{c,x}$	$29.4^{c,x}$	$24.8^{b,x}$	$21.0^{a,x}$	$39.5^{d,y}$	$34.6^{c,y}$	$28.7^{b,y}$	$24.6^{a,y}$
Sat. index	$33.1^{b,x}$	$37.2^{c,x}$	$32.0^{b,x}$	$27.3^{a,x}$	$47.8^{d,y}$	$43.8^{c,y}$	$37.0^{b,y}$	$32.6^{a,y}$
Hue angle	$1.6^{a,y}$	$1.0^{a,x}$	$1.0^{a,x}$	$0.9^{a,x}$	$1.3^{a,x}$	$1.9^{a,y}$	$3.2^{b,y}$	$3.9^{b,y}$

^{abcd}Means within vacuum storage time with same superscripts are not different (P>0.05).

^{xy}Means between storage times at same display time with same superscripts are not different (P>0.05).

Table 5 - Significance levels of the effects of processing ground beef in air and nitrogen on pigment oxygenation, MRA aerobic and anaerobic.

	TRT	T	TRT*T
Pigment oxygenation			
$R_{630}-R_{580}$	n.s.	n.s.	*
Hunter L	n.s.	**	n.s.
Hunter a	n.s.	**	n.s.
Hunter b	n.s.	n.s.	n.s.
Saturation index	n.s.	**	n.s.
Hue angle	n.s.	**	n.s.
MRA, aerobic			
MetMb (%)	n.s.	**	n.s.
MRA, anaerobic			
MetMb (%)	*	**	* (=.054)

**P<0.01
* P<0.05
n.s. P>0.05
TRT = treatment T = time

Table 6 - Effect of processing ground beef in air (A) and nitrogen (N) on pigment oxygenation

Color trait	Vacuum storage (days)	Oxygenation time (min) 0	1	2	3	4	5	8	15	30	60
$R_{630}-R_{580}$	A	$12.6^{e,y}$	$12.5^{d,x}$	$12.8^{f,x}$	$12.4^{c,x}$	$12.4^{c,x}$	$12.3^{b,x}$	$12.3^{b,x}$	$12.3^{b,x}$	$12.4^{c,x}$	$11.6^{a,x}$
	N	$12.5^{a,x}$	$13.2^{d,y}$	$13.2^{d,y}$	$12.8^{c,y}$	$13.2^{d,y}$	$12.8^{c,y}$	$12.7^{b,y}$	$13.9^{g,y}$	$13.8^{f,y}$	$13.7^{e,y}$
Hunter L		28.2^{a}	26.8^{a}	30.0^{ab}	32.1^{abc}	33.8^{bc}	35.8^{cd}	36.9^{cd}	39.6^{d}	39.9^{d}	39.6^{d}
Hunter a		29.9^{de}	31.4^{e}	28.2^{cde}	26.6^{bcd}	25.1^{abc}	23.0^{ab}	21.8^{a}	20.8^{a}	20.7^{a}	21.0^{a}
Hunter b		9.0^{b}	10.3^{b}	9.8^{ab}	9.3^{ab}	8.9^{ab}	8.3^{ab}	7.9^{a}	8.4^{ab}	8.9^{ab}	9.9^{ab}
Saturation index		31.2^{cd}	33.0^{d}	29.8^{cd}	28.2^{bcd}	26.6^{abc}	24.5^{ab}	23.2^{ab}	22.4^{a}	22.5^{a}	23.2^{ab}
Hue angle		3.2^{e}	3.0^{d}	2.8^{cd}	2.8^{cd}	2.8^{cd}	2.7^{cd}	2.7^{c}	2.4^{b}	2.2^{ab}	2.0^{a}

^{abcdef}Means in the same row with same superscript are not different (P>0.05).

^{xy}Means in the same column for $R_{630}-R_{580}$ with the same superscript are not different (P>0.05).

Table 7 - Effect of processing ground beef in air and nitrogen on aerobic metmyoglobin reducing activity, expressed as % MetMb.

Time (hr)	Treatment		Treatment average
	Air	Nitrogen	
0	80.4	77.0	78.7^{a}
2	61.6	58.1	59.8^{bc}
4	62.2	62.1	62.2^{b}
6	59.4	57.4	58.4^{bcd}
8	58.1	57.3	57.7^{bcd}
10	57.2	56.6	56.9^{cd}
12	56.6	58.8	57.7^{bcd}
24	54.2	55.4	54.8^{d}
average	61.2	60.3	

Means with same superscript letter are not different (P>0.05).

All other comparisons are not different (P>0.05).

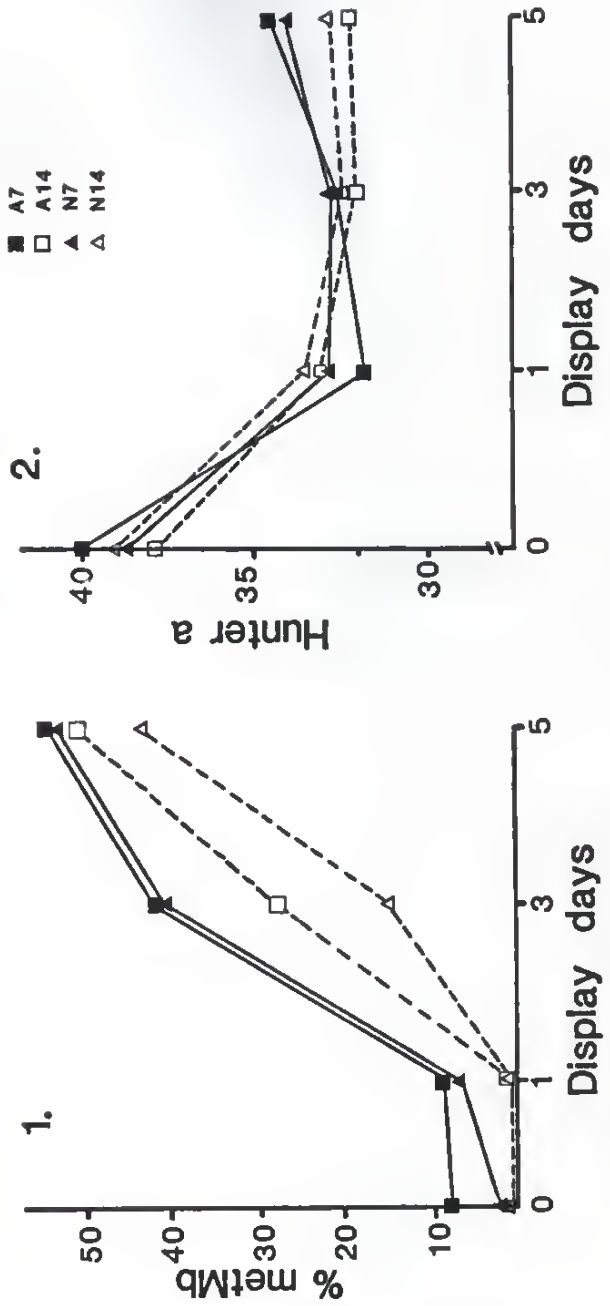
Table 8 - Effect of processing ground beef in air and nitrogen on anaerobic metmyoglobin reducing activity, expressed as % metMb.

Time (min)	Treatment		Treatment average
	Air	Nitrogen	
0	$79.1^{g,x}$	$77.0^{d,x}$	78.1^{g}
1	$67.3^{f,y}$	$55.0^{c,x}$	61.1^{f}
2	$50.8^{e,y}$	$36.2^{b,x}$	43.5^{e}
3	$45.6^{d,e,y}$	$30.6^{ab,x}$	38.1^{d}
4	$39.7^{cd,y}$	$27.6^{a,x}$	33.6^{cd}
5	$36.3^{bc,y}$	$26.6^{a,x}$	31.5^{bc}
8	$26.7^{a,x}$	$25.7^{a,x}$	26.2^{a}
15	$32.4^{ab,y}$	$24.2^{a,x}$	28.3^{ab}
30	$31.1^{ab,x}$	$25.2^{a,x}$	28.1^{ab}
60	$31.0^{ab,x}$	$24.5^{a,x}$	27.8^{ab}
24 hr	$30.2^{ab,x}$	$24.1^{a,x}$	27.1^{ab}
average	43.1^{b}	34.6^{a}	

^{abcdefg}Means with same superscript letter within a column are not different (P>0.05).

^{xy}Means in the same row between treatments with the same superscript are not different (P>0.05).

Figure 1. Metmyoglobin (metMb) and Hunter a values during display at 3° C of ground beef processed in air (A) and nitrogen (N) and stored in vacuum 7 and 14 days.


1.
% metMb
50
40
30
20
10
0
1
3
5
Display days
2.
Hunter a
40
35
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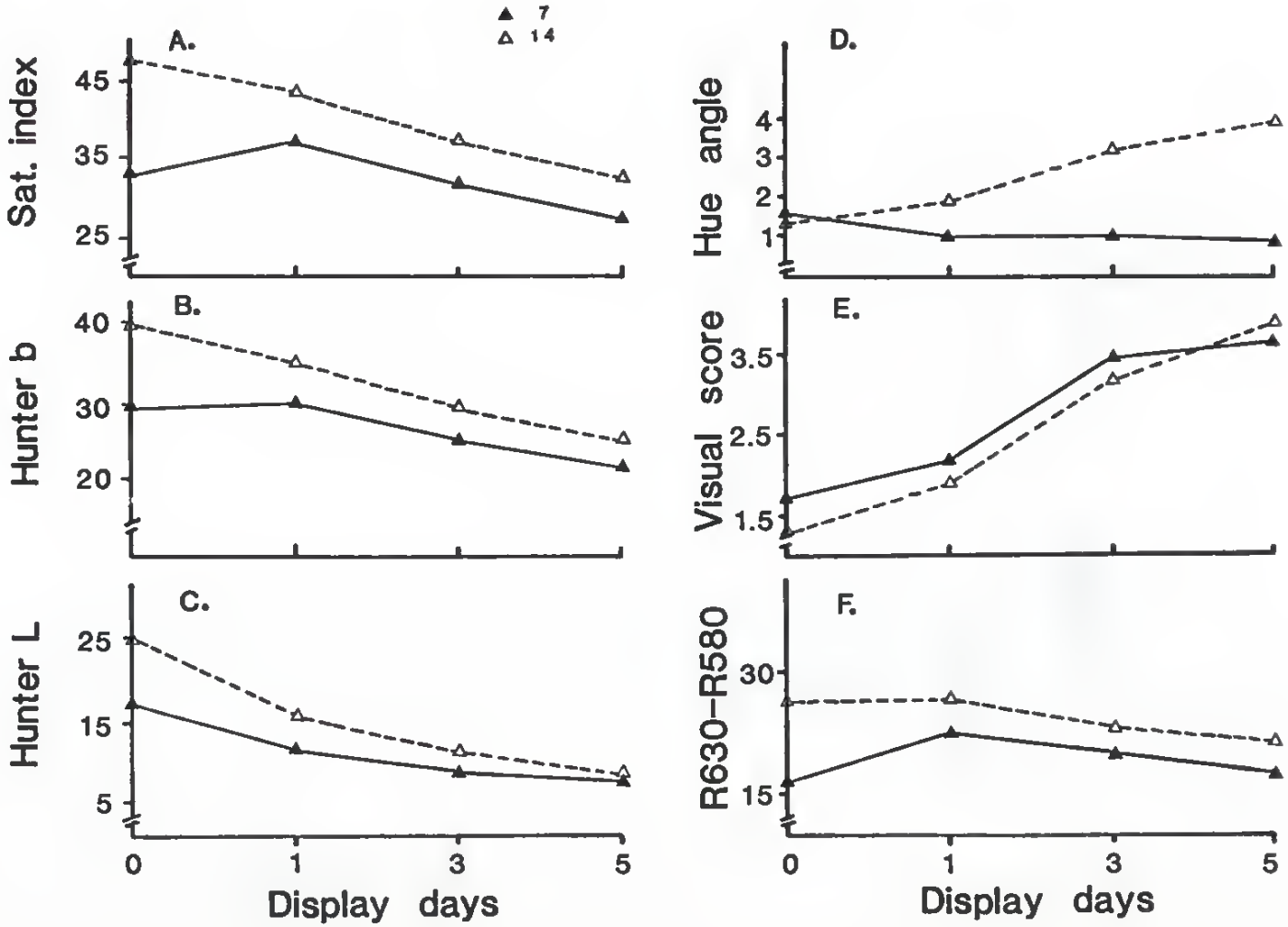
Display days
A7
A14
N7
N14

Figure 2. Color traits of ground beef, stored 7 and 14 days in vacuum, during display at 3° C.


▲ 7
△ 14
A.
Sat. index
45
35
25
B.
Hunter b
40
30
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C.
Hunter L
25
15
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0
1
3
5
Display days
D.
Hue angle
4
3
2
1
E.
Visual score
3.5
2.5
1.5
F.
R630–R580
30
15
0
1
3
5
Display days

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Chapter III

EFFECT OF HOT VERSUS COLD BONING AND VACUUM STORAGE TIME ON GROUND BEEF COLOR STABILITY

Summary

A surface muscle, cutaneous truncii and a deeper muscle, adductor, of 3 beef carcasses were excised 1 hr (H) and 48 hr (C) postmortem and packaged in vacuum 7 and 14 days before evaluation of display color stability (visual score, % metMb, R_{630}-R_{580}, Hunter L, a and b, saturation index and hue angle), anaerobic MRA and pigment oxygenation. Adductor visual scores indicated a rather dark color throughout the display, but were less dark after 5 days display ($p<0.05$) with 14 days vacuum storage. Hunter L values did not change during display for samples stored 7 days, but after 14 days storage were higher from day 3 of display on ($p<0.05$). MetMb of C and H treatment was not different ($p>0.05$). R_{630}-R_{580} tended higher for H. MRA did not show consistent trends and no difference for treatment or storage. During oxygenation, C samples became lighter with decreased Hunter a over time for both 7 and 14 days storage, with no difference between storage times ($p>0.05$). Samples stored 7 days tended toward lower lightness. Saturation index decreased over time and H had higher values than C during most of the oxygenation. MetMb formation was not different between C and H. Up to 15 min oxygenation, C showed higher R_{630}-R_{580} suggesting more oxyMb than H. Cutaneous truncii display visual scores were low (bright red), with C and H not different, except at day 5 when C was higher (darker). No treatment difference was found for Hunter a, R_{630}-R_{580} and saturation index. Seven days storage

caused darker visual scores from day 3 of display on. Hunter a and saturation index were higher on days 3 and 5 of display for samples stored 14 days. Treatment effect of hue angle was significant for C7 ($p<0.05$) but not for C14, with no storage time effect ($p>0.05$). MRA was greatest for C7 and lowest for C14 and H7. $R_{630}-R_{580}$ decreased over time for both C and H; but slightly less for H. During oxygenation, $R_{630}-R_{580}$ changed very little for either 7 or 14 days vacuum storage samples. % metMb increased over time. No treatment effect was found ($p>0.05$) but metMb formation tended to be delayed for H samples. Hunter L was not different ($p>0.05$) between either treatment or vacuum storage time, although C tended lighter than H.

Introduction

Ground beef shares an important portion of the fresh beef market. Understanding and control of the factors that may extend color stability during display in oxygen permeable packages at the retail stores is important and should help to reduce product loss.

Oxygen availability and uptake conditions and temperature are key factors influencing the chemical state of myoglobin. Low temperatures suppress respiratory enzyme activity (Lawrie, 1974) favoring oxygen penetration into the tissue (Snyder, 1964) and consequently deeper pigment oxygenation. When the amount of available oxygen is lowered, either depleted by accelerated enzymatic activity (MacDougall and Taylor, 1975; Bendall, 1972) due to rise in temperature, or at low pO_2 (George and Stratman, 1952; Ledward, 1970), pigment oxidation is favored.

Metmyoglobin (MetMb) is continuously reduced to deoxymyoglobin (deoxyMb) by enzymes present in muscle (Stewart et al., 1965; Ledward, 1970; 1972; Hagler et al., 1979). This activity varies greatly among muscles due to their anatomical location, function and structure which influence the oxygen consumption of the oxidative enzymes and the rate of oxymyoglobin (oxyMb) conversion to deoxyMb. DeoxyMb is readily oxidized to metMb, favoring discoloration, especially when reducing activity is used up (Ledward et al., 1971; Hood, 1980; O'Keeffe and Hood, 1982; Billaut et al., 1984).

Metmyoglobin reducing activity (MRA) declines with time postmortem. Aged meat has poor color stability although it oxygenates faster (Atkinson and Follet, 1973; Ledward et al., 1977; O'Keeffe and Hood, 1980). Prerigor meat is darker, oxygenates less (Conforth and Egbert, 1985) and forms less metMb (Judge and Aberle, 1980). MRA is also less active as a consequence of slow chill rate

(Huffman, 1980; MacDougall, 1982) and grinding (Stewart et al., 1965; Ledward et al., 1977), because of substrate depletion and enzyme dagradation (Urbin and Wilson, 1961; Saleh and Watts, 1968; Bendall, 1972).

Two muscles of different locations on the carcass, shape, functionality and myoglobin content (cutaneous truncii and adductor) were used to evaluate the effect of hot and chilled processing which differ in oxygen exposure during processing, and of 7 versus 14 day vacuum storage time on muscle reducing activity and related characteristics of ground beef.

Materials and Methods

Cutaneous truncii (Ct) and adductor (A) muscles of three young beef cattle (1 Hereford and 1 Simmental steer and 1 Brahman x Hereford heifer) were excised from the left side at 1 hr post mortem (hot boned, H) and at 48 hr from the right side (conventionally chilled, C). The pH decline of both muscles was measured at 1, 8 and 24 hr using a probe electrode. The 24 hr pH ranged from 5.71 to 5.90 for the adductor and 5.86 to 6.21 for the cutaneous truncii. Excess fat was trimmed and the meat coarsely ground (12.7 mm plate), mixed, and samples were taken randomly, vacuum packaged and stored for either 7 or 14 days in the dark at 2 to 3° C. Coarse ground samples for pigment oxygenation and MRA studies were formed into patties and wrapped in polyvinylchloride (PVC) film prior to vacuum overwrap packaging. After storage, samples used for display were reground through a 3.2 mm plate and repackaged in PVC film.

Display Color Stability

After vacuum storage for 7 and 14 days, samples were reground through a 3.2 mm plate (Oster grinder), formed into patties and rewrapped in PVC film. Two patties of each treatment were allowed to oxygenate one hour before first color evaluation. Display study was conducted in an open top case under 970 lux Natural fluorescent lighting for 24 hr per day at 2 to 4° C for 5 days. Coded samples randomly distributed were evaluated visually by a 5 member panel for overall lean color using the KSU 5 point scale (Kropf et al., 1971) to the nearest 0.5 (1 = very bright red, 2 = bright red, 3 = slightly dark red, 4 = dark red or brown, 5 = extremely dark red or brown) at the beginning of display (time 0) and at 1, 3 and 5 days of display. Spectrophotometric reflectance measurement taken at the same intervals included those at 525, 572, 580 and 630 nm and Hunter L, a and b values (Hunter, 1958) for Illuminant A were taken with a Hunterlab D-54 reflectance spectrophotometer.

Hunter a and b data were also converted to hue angle ($\tan^{-1}$ b/a) and saturation index $[(a^2 + b^2)^{\frac{1}{2}}]$. These values along with L are the recommended color attributes to specify a color using the HunterLab system (Setser, 1978).

Metmyoglobin Reducing Activity

MRA was determined by an aerobic method using a modification from Ledward (1972) as described by Sleper et al., (1983). At 7 and 14 days of vacuum storage, four patties of each muscle treatment combination (AH7, AH14, AC7, AC14, CtH7, CtH14, CtC7 and CtC14) were removed from the vacuum package, excess moisture on the PVC wrap wiped off and patties placed in an anaerobic incubator with a 1% oxygen environment for 48 hr at 3 to 4° C, to induce metMb formation. The incubator was evacuated and flushed several times with a gas

mixture of 1% oxygen and 99% nitrogen. Gas samples taken from the incubator were analysed with the MOCON instrument to monitor the oxygen content. Each sample was removed from the incubator (time 0) under continous gas mixture flushing to maintain proper atmosphere in the incubator and scanned spectrophotometrically at two different locations for percentage reflectance at 525 and 572 nm. MRA in an oxygen permeable package was determined by calculating % metMb at 2, 4, 8 and 24 hr air exposure, at 3 to 4° C.

Pigment Oxygenation

Five patties of each treatment, at 7 and 14 days of vacuum storage were used in this study. Percentage reflectance at 580 and 630 nm and Hunter L, a and b values (1958) for Illuminant A were measured immmediately after opening the vacuum package (time 0) and after 5, 10, 15, 30 and 60 minutes at 3 different locations). Samples were scanned through the PVC film and kept at room temperature (24° C) during evaluation. The reflectance difference at 580 and 630 nm ($R_{630} - R_{580}$) was used as an indicator of redness.

Calculation of MetMb

Concentration of metMb was calculated from K/S ratios as described in Chapter II.

Total Pigment

Total pigment concentration was determined by Hornsey's acidified acetone procedure (1956) and ppm haematin was converted to mg/g wet weight applying the conversion factor (0.026) used by Franke and Solberg (1971).

Statistical Analysis

Data were analysed by analysis of variance procedures for a multi split-plot design and means were compared by LSD test (SAS Institute, 1979; Cochran and Cox, 1957).

Results and Discussion

The total heme pigment concentration of the adductor muscle muscles from the three carcasses was 7.91, 6.35 and 7.99 and for the cutaneous truncii was 2.97, 2.24 and 2.22 mg/g.

Adductor Muscle

Table 1 summarizes the results of analysis of variance of adductor muscle (A) color traits, including a 5 day display study, aerobic metMb reducing activity (MRA) and rate of oxygenation.

Display effects. The interaction of vacuum storage time and display time (ST*T) was significant ($p<0.05$) for visual score, % metMb, Hunter a and b values, saturation index and hue angle (Table 1). MetMb % increased over display time for both A7 and A14 samples ($p<0.05$). The trend of the A7 samples was for a higher metMb formation rate (Table 2; Fig. 1) than for A14 samples.

Adductor visual scores indicated rather dark color from the beginning of the display. These became more discolored over display time for both A7 and A14 samples but A14 beef was less dark at the end of display ($p<0.05$) (Table 2; Fig. 2).

Hunter L values for A7 remained unchanged ($p<0.05$) from day 1 to day 5 of display and for A14 were unchanged from day 3 to 5. Samples stored 14 days

started out with higher L values but had lower lightness at day 5 ($p<0.05$) (Table 2; Fig. 2).

Hunter a values slowly decreased over time for A7 ($p<0.05$), but A14 had no change in values throughout the display ($p<0.05$). Samples stored in vacuum 14 days had higher a values than those stored for 7 days from the 3rd day of display on ($p<0.05$) (Table 2; Fig. 2). A14 samples tended to increase in Hunter b values with time and A14 had higher b values than A7 at day 3 and 5 of display.

Saturation index had opposite trends. A7 decreased and A14 increased. Hue angle decreased over display time, with no significant storage time effect.

A treatment, chilled (C) versus hot boning (H), versus display time interaction (CH*T) was calculated for metMb, R_{603}-R_{580} and hue angle ($p<0.05$) (Table 1). H samples formed less metMb at 0 and 1 day of display, but more at 3 and 5 days than C samples (Table 2; Fig. 1). Redness, as expressed by the difference between % R at 630 nm and 580 nm, decreased ($p<0.05$) up to day 3 of display for both treatments. C samples tended to be redder than the H throughout the display, but the difference was not significant ($p>0.05$) (Table 2).

Hue angle of C samples decreased up to day 3 of display but practically remained unchanged for the H treatment at day 1, 3 and 5 of display. Overall treatment means were not different ($p>0.05$).

A treatment versus vacuum storage time interaction (CH*ST) was calculated for Hunter L (Table 1). C samples from both 7 and 14 days vacuum storage had higher L values ($p<0.01$) (Table 3), but no vacuum storage time effect was found for either C or H samples.

Metmyoglobin reducing activity. A three way interaction ($p<0.01$) was found between treatment, vacuum storage time and time of reduction in air (CH*ST*T)

(Table 1). The means did not show consistent trends. Overall, there was not much difference between C and H treatments at either 7 or 14 days storage except at 1 hr (Table 4). Since adductor muscle is located deeper in the carcass, its exposure to oxygen while remaining on the carcass is minimal and little difference was expected between treatments.

Pigment oxygenation. A three way interaction of C versus H treatments, vacuum storage time and time of oxygenation (CH*ST*T) was calculated ($p<0.01$) for Hunter L, a and b values, saturation index and hue angle (Table 1). C samples became lighter (higher L value) over time ($p<0.05$), both from 7 and 14 days storage, but lightness was not different when storage times were compared ($p<0.05$). Hunter L values of H14 treatment increased faster during the first 5 min and then was slower up to 60 min. Compared to C, H samples had lower L values, ie., were darker at both storage times. Samples stored 7 days (C7 and H7) tended to have lower lightness, but were not different after 60 min.

Comparing C and H from either 7 or 14 days storage, H samples showed lower L values ($p<0.05$), ie., were darker, as expected, but the difference was smaller for samples stored 14 days (Table 5; Fig. 4).

Hunter a values decreased over time for both C and H treatments at either 7 or 14 days. C7 and C14 were not different, whereas H14 had a fast decrease during the first 5 min and then remained practically unchanged, following the opposite trend as L value (Table 5; Fig. 4). Hunter a values were higher for samples stored 7 days than for the 14 day samples, this difference being significant only during the first 15 min of oxygenation.

Hunter b values were not different over time for either C7 or C14 ($p>0.05$). H7 values were higher with more variation than for H14 (Table 6).

Saturation index decreased over time up to 10 min of oxygenation ($p<0.05$) for both C and H treatments. C means were not different for 7 and 14 days storage ($p>0.05$). From 7 day storage samples, C had lower saturation index during the first 10 min; thereafter the means were not different ($p>0.05$). However, from 14 days H tended to have lower means (Table 5).

Hue angle decreased ($p<0.05$) over time for either treatment. Storage time effect was not different for C. H14 samples tended to have higher hue angle than H7 samples. With 7 days storage, H means did not follow a constant pattern, and were different (higher) only at 15 and 60 min (Table 5).

Interaction ($p<0.01$) of treatment (C versus H) versus oxygenation time (CH*T) was found for redness ($R_{630}-R_{580}$). For % metMb there was a significant interaction ($p<0.05$) of vacuum storage time versus oxygenation time (ST*T) (Table 1).

Seven day storage (A7) showed a trend of forming less metMb during the pigment oxygenation study than A14, but the difference was not significant (Table 6; Fig. 3). Up to 15 min oxygenation, C samples had higher $R_{630}-R_{580}$, ie., were brighter red than H samples. For both C and H treatments redness did not increase after 5 min, and started decreasing at 30 min (Table 6).

Cutaneous truncii Muscle

Table 7 summarizes the results of analysis of variance of cutaneous truncii (Ct) color traits, including a 5 day display study, aerobic metMb reducing activity (MRA) and rate of oxygenation.

Display effects. A three way interaction of treatment, vacuum storage time and display time (CH*ST*T) was found ($p<0.01$) for Hunter b values (Table 7).

Hunter b tended to increase over time, except for C7 samples, where the means tended to decrease but were not different from the day 1 of display on ($p<0.05$) (Table 8). C and H treatments, of either 7 or 14 days vacuum storage were not different except on day 0 of display when C7 was higher. Means of C7 and C14 were not different, except on day 5 of display, when C14 was higher ($p<0.05$). H7 means were higher than H14 means throughout the entire display time ($p<0.05$). Hunter b is of limited value in defining meat color.

A treatment (C versus H) x display time interaction (CH*T) was calculated ($p<0.05$) for visual score, $R_{630}-R_{580}$, Hunter L and a and saturation index (Table 7).

Visual scores were low at the beginning of the display indicating bright red color and then the scores increased over time for both C and H treatments. The means between C and H were not different, except for the last day of display when C had darker color (higher score) (Table 8; Fig. 2).

Hunter a, $R_{630}-R_{580}$ and saturation index decreased over time ($p<0.05$) except for saturation index of H samples which were not different ($p>0.05$) over time. For all traits, there was no overall difference ($p>0.05$) between treatments, but H samples tended to have higher values. This difference was significant ($p<0.05$) at day 3 and 5 of display for Hunter a values.

Visual score, Hunter a and saturation index had a vacuum storage time versus display time interaction ($p<0.05$) (Table 7). Visual scores indicated darker color at days 3 and 5 of display for 7 day samples (Table 8; Fig. 2). Hunter L did not change over time ($p<0.05$). Hunter a from 7 and 14 days vacuum storage (Table 8; Fig. 2) and saturation index from 7 day samples decreased over time. They were higher on day 3 and 5 of display for 14 days storage than for 7 day storage.

Interaction of C and H treatments versus vacuum storage time was

significant ($p<0.05$) for hue angle (Table 7). Hue angle was different at 7 days between C and H samples ($p<0.05$) but not for the 14 day storage samples. Means between storage time were not different for either C or H samples (Table 9).

Table 10 shows that % metMb formed on the sample surface during display increased ($p<0.05$) with display time (Fig. 1), with no significant treatment or vacuum storage time effect.

Metmyoglobin reducing activity. An interaction ($p<0.01$) of CH*ST*T was found for aerobic MRA (Table 7). C7 samples formed highest % metMb during incubation in 1% oxygen environment, followed by C14 (Table 11). H7 and H14 formed about half of that of C7 which also showed greatest reducing activity because after 24 hr approximately 50% of the initial metMb concentration was reduced. About $\frac{1}{4}$ of the total metMb was lost between 1 and 24 hr for hot, 14 day storage sample.

Pigment oxygenation. An interaction of CH*ST*T was found ($p<0.05$) for saturation index determined during oxygenation (Table 7). The saturation index decreased over time for H7 and H14 and for C14. For C7 it remained practically unchanged. At either 7 or 14 days storage the treatment effect was not significant. C samples were not different between 7 and 14 days storage time. H7 samples had higher values only up to the first 5 min; thereafter, the means were not different (Table 12).

$R_{630}-R_{580}$ had interactions ($p<0.01$) of both treatment (C or H) and storage time with display time (Table 7), but consistent and meaningful trends were not found for this measurement which should be related to oxyMb concentration and to bright red color.

A significant treatment (C or H) versus oxygenation time interaction (CH*T) was found for metMb, Hunter L, a and b values ($p<0.01$) (Table 7). MetMb % of both C and H increased over time. Although the C samples tended to form more metMb (less MRA), there was no significant ($p>0.05$) treatment effect (Table 12; Fig. 3).

Hunter L and b values increased with oxygenation time for either C or H. Lightness was not different ($p>0.05$) between treatments, but C tended to have higher means (lighter). After 30 min oxygenation, Hunter b values of C were higher ($p<0.05$) (Table 12; Fig. 4).

A CH*ST interaction ($p<0.05$) was found for Hunter L (Table 7). C7 and C14 had slightly higher values than H7 and H14 but the difference was not significant. Me ans btween vacuum storage time for either C or H were not different ($p>0.05$) (Table 13).

Hue angle during oxygenation, averaged over C and H treatments and vacuum storage time, decreased with time.

In the display study, the ground adductor muscle packaged in PVC was dark in color initially and remained dark during the 5 days of display. The cutaneous truncii showed a more desirable color early in display and relatively low metMb levels on the surface compared to the adductor. No well defined effect of hot compared to cold processing was found for the adductor. After five days of display, cutaneous truncii processed chilled after remaining on carcass 48 hr had a darker color than if hot boned and processed. For each muscle, the sample vacuum stored 14 days showed some indications of more desirable traits than those vacuum stored for 7 days.

Aerobic MRA determinations for the adductor were inconclusive and did not show treatment or storage effects. Cutaneous truncii MRA studies showed a higher

metMb level initially and after 24 hr aerobic exposure for cold processed compared to hot processed.

Results of pigment oxygenation studies are largely inconclusive and do not relate strongly to display and MRA results. The cold processed adductor showed a higher R_{630}-R_{580} ratio than for hot processed, but other traits were not clearly affected. Storage for 14 day with adductor samples resulted in higher metMb at 5 min or longer oxygenation times. For cutaneous truncii muscle, the hot processed samples tended to have less metMb early in the oxygenation study.

We expected no advantages for hot processing of the adductor muscle, because it has a very limited exposure to oxygen while on the intact carcass. Therefore, removing it hot in order to reduce oxygen exposure had little advantage. We expected to see a more dramatic and consistent advantage for removing the cutaneous truncii hot and minimizing its exposure to oxygen. However we only had trends and limited evidence that this was true.

Table 1 - Significance levels of effects of hot vs. chilled boning, 7 and 14 days vacuum storage and display or test times, on ground adductor muscle color stability.

	CH	ST	T	CH*T	ST*T	CH*ST	CH*ST*T
Display							
Visual score	n.s.	n.s.	**	n.s.	**	n.s.	n.s.
MetMb (%)	n.s.	n.s.	**	*	*	n.s.	n.s.
R_{630}-R_{580}	n.s.	*	**	**	n.s.	n.s.	n.s.
Hunter L	*	n.s.	**	n.s.	n.s.	**	n.s.
Hunter a	n.s.	*	**	n.s.	**	n.s.	n.s.
Hunter b	n.s.	**	**	n.s.	**	n.s.	n.s.
Saturation index	n.s.	*	n.s.	n.s.	**	n.s.	n.s.
Hue angle	n.s.	n.s.	n.s.	*	*	n.s.	n.s.
MRA							
MetMb (%)	n.s.	n.s.	**	**	**	n.s.	**
Rate of oxygenation							
R_{630}-R_{580}	n.s.	n.s.	**	**	n.s.	n.s.	n.s.
MetMb (%)	n.s.	n.s.	**	n.s.	*	n.s.	n.s.
Hunter L	*	**	**	**	**	**	**
Hunter a	n.s.	*	**	**	**	*	**
Hunter b	n.s.	n.s.	**	**	**	*	**
Saturation index	n.s.	*	**	**	**	*	**
Hue angle	n.s.	n.s.	**	**	**	n.s.	**

***P<0.01
**P<0.05
n.s.P>0.05
CH = treatment (chilled x hot boning)
ST = vacuum storage time (7 x 14 days)
T = time

Table 2 - Effects of hot or chilled boning, after 7 and 14 days vacuum storage, on ground adductor muscle color stability during display at 3°C.

Color trait	Vacuum storage (days)	Display (days) 0	1	3	5
MetMb (%)	7	$7.6^{a,x}$	$31.8^{b,x}$	$84.8^{c,y}$	$79.3^{c,x}$
	14	$11.0^{a,x}$	$27.7^{a,x}$	$68.8^{b,x}$	$83.0^{b,x}$
Visual score	7	$2.8^{a,x}$	$3.3^{b,x}$	$4.4^{c,x}$	$4.8^{d,y}$
	14	$2.9^{a,x}$	$3.3^{b,x}$	$4.2^{c,x}$	$4.3^{c,x}$
Hunter a	7	$25.1^{b,x}$	$25.1^{b,x}$	$18.8^{a,x}$	$17.6^{a,x}$
	14	$24.9^{a,x}$	$24.7^{a,x}$	$23.4^{a,y}$	$23.0^{a,y}$
Hunter b	7	$13.7^{a,x}$	$19.5^{b,x}$	$20.4^{b,x}$	$17.8^{ab,x}$
	14	$13.3^{a,x}$	$18.9^{b,x}$	$23.9^{b,y}$	$26.1^{b,y}$
Saturation index	7	$28.6^{b,x}$	$31.8^{b,x}$	$27.8^{ab,x}$	$25.0^{ab,x}$
	14	$28.2^{a,x}$	$31.1^{ab,x}$	$33.5^{ab,y}$	$34.9^{b,y}$
Hue angle	7	$1.7^{c,x}$	$1.0^{b,x}$	$0.5^{a,x}$	$0.6^{a,x}$
	14	$1.7^{c,x}$	$1.0^{ab,x}$	$0.6^{ab,y}$	$0.5^{a,x}$
	treatment				
MetMb	C	$12.5^{a,x}$	$34.3^{b,x}$	$72.7^{c,x}$	$78.1^{c,x}$
	H	$6.0^{a,x}$	$25.2^{b,x}$	$80.9^{c,x}$	$84.2^{c,x}$
$R_{630-580}$	C	$14.5^{c,x}$	$9.0^{b,x}$	$5.2^{a,x}$	$4.6^{a,x}$
	H	$10.8^{c,x}$	$7.0^{b,x}$	$3.5^{a,x}$	$3.2^{a,x}$
Hue angle	C	$1.6^{c,x}$	$1.0^{b,x}$	$0.6^{a,x}$	$0.6^{a,x}$
	H	$1.8^{c,x}$	$1.0^{a,x}$	$0.5^{a,x}$	$0.5^{a,x}$

^{abcd}Means in same row with the same superscript are not different (P>0.05).

^{xy}Means between treatments or storage time within display day with same superscript are not different (P>0.05).

C = chilled, H = hot boned

Table 3 - Effects of hot or chilled boning, after 7 and 14 days vacuum storage, on Hunter L values of ground adductor muscle during display at 3°C.

Vacuum Storage (days)	Treatment	
	Chilled	Hot boned
7	$33.4^{b,x}$	$26.6^{a,x}$
14	$33.3^{b,x}$	$27.9^{a,x}$

^{ab}Means with the same superscript between treatments are not different (P>0.05).

^{x}Means between storage time with same superscripts are not different (P>0.05).

Table 4 - Effects of hot or chilled boning of ground adductor muscle, after 7 and 14 days vacuum storage, on % MetMb reduction at 3°C.

Treatment traits	Vacuum storage (days)	Time (h)				
		1	2	4	8	24
Chilled	7	$61.7^{a,m,x}$	$90.4^{c,m,x}$	$84.7^{c,m,x}$	$72.9^{b,m,x}$	$66.4^{a,m,x}$
Chilled	14	$89.4^{b,m,y}$	$89.4^{b,m,x}$	$84.9^{b,m,x}$	$82.1^{b,m,x}$	$72.2^{a,m,x}$
Hot boned	7	$90.1^{c,n,x}$	$83.2^{b,m,x}$	$79.1^{ab,m,x}$	$72.2^{a,m,x}$	$78.5^{ab,m,x}$
Hot boned	14	$91.4^{c,m,x}$	$95.7^{d,m,x}$	$86.1^{bc,m,x}$	$83.1^{b,m,x}$	$71.9^{a,m,x}$

^{abc}Means in the same row with same superscript are not different (P>0.05).

^{xy}Means between storage time within treatment with same superscript are not different (P>0.05).

^{mn}Means with same superscripts between treatments are not different (P>0.05).

Table 5 - Effects of hot or. chilled boning of ground adductor muscle, after 7 and 14 days vacuum storage, during oxygenation.

Color trait	Treatment	Vacuum storage (days)	Time (min) 0	5	10	15	30	60
Hunter L	C	7	$29.3^{a,n,x}$	$36.2^{b,n,x}$	$40.1^{c,n,x}$	$41.5^{c,n,x}$	$40.5^{c,n,x}$	$41.0^{c,n,x}$
	C	14	$29.3^{a,n,x}$	$36.2^{b,n,x}$	$40.4^{c,m,x}$	$41.2^{c,n,x}$	$41.7^{c,m,x}$	$40.7^{c,m,x}$
	H	7	$18.7^{a,m,x}$	$21.3^{b,m,x}$	$26.5^{c,m,x}$	$33.5^{d,m,x}$	$31.2^{d,m,x}$	$34.1^{e,m,i}$
	H	14	$20.5^{a,m,x}$	$32.2^{b,m,y}$	$36.2^{c,m,y}$	$35.7^{c,m,x}$	$36.8^{c,m,y}$	$36.2^{c,m,x}$
Hunter a	C	7	$32.0^{d,m,x}$	$26.4^{c,m,x}$	$23.7^{b,m,x}$	$22.3^{ab,m,x}$	$22.5^{ab,m,x}$	$20.5^{a,m,x}$
	C	14	$32.3^{c,m,x}$	$27.0^{b,n,x}$	$23.4^{a,n,x}$	$22.7^{a,m,x}$	$21.9^{a,m,x}$	$20.8^{a,m,x}$
	H	7	$37.0^{d,n,x}$	$35.0^{d,n,y}$	$29.1^{c,n,x}$	$21.6^{ab,m,x}$	$23.9^{b,m,y}$	$19.8^{a,m,x}$
	H	14	$34.7^{c,m,x}$	$23.2^{b,m,x}$	$20.4^{a,m,x}$	$21.0^{a,m,x}$	$19.5^{a,m,x}$	$18.7^{a,m,x}$
Hunter b	C	7	$10.1^{a,m,x}$	$10.7^{a,m,x}$	$10.2^{a,m,x}$	$9.8^{a,m,x}$	$10.4^{a,m,x}$	$10.3^{a,n,x}$
	C	14	$10.6^{a,m,x}$	$11.4^{a,n,x}$	$10.1^{a,n,x}$	$10.1^{a,m,x}$	$10.4^{a,n,x}$	$10.8^{a,n,x}$
	H	7	$11.8^{bc,m,x}$	$15.9^{d,n,y}$	$13.5^{c,n,y}$	$8.2^{a,m,x}$	$10.9^{b,m,y}$	$8.0^{a,m,x}$
	H	14	$11.1^{b,m,x}$	$8.5^{a,m,x}$	$8.0^{a,m,x}$	$8.6^{a,m,x}$	$8.1^{a,m,x}$	$8.0^{a,m,x}$
Saturation index	C	7	$33.6^{c,m,x}$	$28.5^{b,m,x}$	$25.8^{ab,m,x}$	$24.4^{a,m,x}$	$24.8^{a,m,x}$	$23.0^{a,m,x}$
	C	14	$34.0^{c,m,x}$	$29.3^{b,n,x}$	$25.5^{a,n,x}$	$24.8^{a,m,x}$	$24.2^{a,n,x}$	$23.5^{a,m,x}$
	H	7	$38.8^{d,n,i}$	$38.5^{d,n,y}$	$32.1^{c,n,y}$	$23.1^{a,m,x}$	$26.2^{b,m,y}$	$21.4^{a,m,x}$
	H	14	$36.4^{c,m,x}$	$24.7^{b,m,x}$	$21.9^{ab,m,x}$	$22.6^{ab,m,x}$	$21.1^{a,m,x}$	$20.3^{a,m,x}$
Hue angle	C	7	$3.1^{c,m,x}$	$2.3^{b,n,x}$	$2.2^{b,n,x}$	$2.2^{b,m,x}$	$2.0^{a,m,x}$	$1.8^{a,m,x}$
	C	14	$3.0^{c,m,x}$	$2.2^{b,m,x}$	$2.2^{b,m,x}$	$2.1^{b,m,x}$	$2.0^{ab,m,x}$	$1.8^{a,m,x}$
	H	7	$3.1^{d,m,x}$	$2.1^{ab,m,x}$	$2.0^{a,m,x}$	$2.5^{c,n,x}$	$2.0^{a,m,x}$	$2.4^{b,n,x}$
	H	14	$3.0^{c,m,x}$	$2.6^{b,n,y}$	$2.4^{ab,n,y}$	$2.3^{a,n,x}$	$2.3^{a,n,y}$	$2.2^{a,m,x}$

^{abcd}Means with the same superscript in a row are not different (P>0.05).

^{mn}Means of treatments within vacuum storage time and oxygenation time, with same superscript, are not different (P>0.05).

^{xy}Means of vacuum storage time within treatment and oxygenation time, with same superscript, are not different (P>0.05).

Table 6 - Effects of hot or chilled boning of ground adductor muscle, after 7 and n6 days vacuum storage, during oxygenation.

Color traits	Treatment	Vacuum storage (days)	Time (min) 0	5	10	15	30	60
$R_{630}-R_{580}$	C		$14.3^{b,y}$	$15.1^{c,y}$	$15.2^{c,y}$	$15.0^{c,y}$	$14.3^{b,x}$	$12.2^{a,x}$
	H		$10.5^{b,x}$	$11.1^{bc,x}$	$11.2^{c,x}$	$11.2^{c,x}$	$10.9^{b,x}$	$9.8^{a,x}$
MetMb (%)		7	$8.4^{a,x}$	$1.9^{a,x}$	$6.4^{a,x}$	$15.6^{a,x}$	$13.4^{a,x}$	$23.8^{b,x}$
		14	$0.5^{a,x}$	$7.1^{ab,x}$	$17.4^{bc,y}$	$18.7^{bc,x}$	$19.0^{bc,x}$	$26.9^{c,x}$

^{abc}Means with same superscript in a row are not different (P>0.05).

^{xy}Means with same superscript between treatment or vacuum storage time within oxygenation time are not different (P>0.05).

C = chilled, H = hot boned.

Table 7 - Significance levels of effects of hot or chilled boning, after 7 and 14 days vacuum storage, on ground cutaneous truncii muscle color stability.

	CH	ST	T	CH*T	ST*T	CH*ST	CH*ST*T
Display							
Visual score	n.s.	*	**	**	**	n.s.	n.s.
MetMb (%)	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.
R_{630}-R_{580}	n.s.	*	**	**	n.s.	n.s.	n.s.
Hunter L	n.s.	n.s.	**	n.s.	**	n.s.	n.s.
Hunter a	n.s.	*	**	*	* (5.3)	n.s.	n.s.
Hunter b	n.s.	*	**	**	*	n.s.	**
Saturation index	n.s.	**	***	***	***	n.s.	n.s.
Hue angle	*	*	**	n.s.	n.s.	*	n.s.
MRA							
MetMb (%)	*	*	**	**	**	*	**
Rate of oxygenation							
R_{630}-R_{580}	n.s.	n.s.	**	**	**	n.s.	n.s.
MetMb (%)	n.s.	n.s.	**	**	n.s.	n.s.	n.s.
Hunter L	n.s.	*	**	**	n.s.	*	n.s.
Hunter a	n.s.	n.s.	**	**	n.s.	n.s.	n.s.
Hunter b	n.s.	*	**	**	n.s.	n.s.	n.s.
Saturation index	n.s.	n.s.	**	**	n.s.	n.s.	*
Hue angle	n.s.	*	**	n.s.	n.s.	n.s.	n.s.

***P<0.01
**P<0.05
n.s. P>0.05
CH = treatment (chilled x hot boning)
ST = vacuum storage time (7 x 14 days)
T = time

Table 8 - Effects of hot or chilled boning after 7 and 14 days vacuum storage, and display time, on ground cutaneous truncii color stability during display at 3°C.

Color trait	Treatment or storage (days)		Display (days) 0	1	3	5
Visual score	C		$1.5^{a,x}$	$2.0^{a,x}$	$3.0^{b,x}$	$3.8^{c,y}$
	H		$1.6^{a,x}$	$1.9^{a,x}$	$2.6^{b,x}$	$3.3^{c,x}$
$R_{630}-R_{580}$	C		$21.3^{c,x}$	$15.7^{b,x}$	$9.0^{a,x}$	$5.8^{a,x}$
	H		$18.0^{c,x}$	$15.0^{bc,x}$	$11.9^{ab,x}$	$8.9^{a,x}$
Hunter a	C		$26.4^{c,x}$	$22.6^{bc,x}$	$17.8^{ab,x}$	$14.4^{a,x}$
	H		$23.9^{b,x}$	$22.3^{ab,x}$	$20.9^{ab,y}$	$17.7^{a,y}$
Saturation index	C		$30.4^{c,x}$	$26.4^{bc,x}$	$23.1^{ab,x}$	$20.0^{a,x}$
	H		$26.8^{a,x}$	$26.2^{a,x}$	$25.9^{a,x}$	$23.0^{a,x}$
Visual score	7		$1.6^{a,x}$	$1.9^{a,x}$	$3.1^{b,y}$	$4.0^{c,y}$
	14		$1.6^{a,x}$	$2.0^{a,x}$	$2.5^{b,x}$	$3.1^{c,x}$
Hunter L	7		$45.8^{a,x}$	$47.4^{a,y}$	$44.5^{a,x}$	$45.9^{a,y}$
	14		$48.1^{b,y}$	$44.7^{a,x}$	$43.5^{a,x}$	$43.4^{a,x}$
Hunter a	7		$24.1^{c,x}$	$21.1^{b,x}$	$16.2^{ab,x}$	$12.7^{a,x}$
	14		$26.3^{b,x}$	$23.8^{ab,x}$	$22.5^{ab,y}$	$19.4^{a,y}$
Saturation index	7		$27.3^{c,x}$	$24.7^{bc,x}$	$21.2^{ab,x}$	$18.0^{a,x}$
	14		$29.8^{a,x}$	$27.8^{a,x}$	$27.7^{a,y}$	$25.3^{a,y}$
	storage*treatment					
Hunter b	7	C	$15.6^{b,n,s}$	$13.0^{a,m,s}$	$13.4^{a,m,s}$	$12.3^{a,m,s}$
	7	H	$10.2^{a,m,s}$	$12.5^{b,m,s}$	$13.8^{b,m,s}$	$12.8^{b,m,s}$
	14	C	$14.3^{a,m,s}$	$14.3^{a,m,s}$	$15.5^{a,m,s}$	$15.4^{a,m,t}$
	14	H	$13.8^{a,m,t}$	$14.8^{ab,m,t}$	$16.4^{c,m,t}$	$16.1^{b,m,t}$

^{abcd}Means in same row with the same superscript letter are not different (P>0.05).
^{mn}Means of treatments within vacuum storage time in a column with same superscript are not different (P>0.05).
^{xy}Means of vacuum storage time or treatment for any color trait with same superscript in a column are not different (P>0.05).
stMean of vacuum storage time within a treatment in a column with same superscript are not different (P>0.05).

Table 9 - Effects of hot or chilled boning, after 7 and 14 days vacuum storage, on hue angle of ground cutaneous truncii muscle during display at 3°C.

Vacuum Storage (days)	Treatment	
	Chilled	Hot boned
7	$1.0^{a,x}$	$1.3^{b,x}$
14	$1.2^{a,x}$	$1.3^{a,x}$

^{ab}Means of treatments with same superscript are not different (P>0.05).

^{x}Means in a column with same superscript are not different (P>0.05).

Table 10 - Metmyoglobin of ground cutaneous truncii muscle during display at 3°C.

Display (days)	% MetMb (%)
0	2.5[a]
1	17.8[b]
3	32.5[c]
5	46.6[d]

[abcd]Means with different superscript are significant ($P<0.05$).

Table 11 - Effects of hot vs. chilled boning of ground cutaneous truncii muscle, at 7 and 14 days vacuum storage, on % MetMb reduction at 3°C.

Treatment	Vacuum storage (days)	Time (h) 1	2	4	8	24
Chilled	7	$95.5^{c,n,y}$	$59.8^{b,n,x}$	$54.3^{ab,n,x}$	$54.9^{ab,n,x}$	$47.5^{a,n,x}$
Chilled	14	$68.6^{b,n,x}$	$67.1^{ab,n,x}$	$63.9^{ab,n,x}$	$60.2^{a,m,x}$	$60.4^{a,n,y}$
Hot boned	7	$45.5^{b,m,x}$	$34.9^{a,m,x}$	$31.6^{a,m,x}$	$31.5^{a,m,x}$	$32.0^{a,m,x}$
Hot boned	14	$57.7^{b,m,y}$	$56.3^{b,m,y}$	$51.5^{b,m,y}$	$55.4^{b,m,y}$	$43.3^{a,m,y}$

^{abc}Means with same superscript letter in a row are not different (P>0.05).

^{xy}Means of vacuum storage time with same supercript within treatments are not different (P>0.05).

^{mn}Means of treatments with same superscript within vacuum storage time are not different (P>0.05).

Table 12 - Effects of hot or chilled boning of ground cutaneous truncii muscle, after 7 and 14 days vacuum storage during oxygenation.

Color Traits	Treatment		Time (min) 0	5	10	15	30	60
$R_{630}-R_{580}$		C	$14.6^{a,x}$	$15.8^{bc,x}$	$16.0^{bc,x}$	$16.5^{c,x}$	$16.6^{c,x}$	$15.2^{ab,x}$
		H	$15.7^{b,x}$	$16.6^{bc,x}$	$16.9^{c,x}$	$16.8^{c,x}$	$16.0^{bc,x}$	$14.7^{a,x}$
MetMb (%)		C	$14.0^{a,x}$	$15.4^{a,x}$	$15.5^{a,x}$	$15.3^{a,x}$	$16.3^{a,x}$	$19.8^{a,x}$
		H	$1.4^{a,x}$	$1.7^{a,x}$	$6.4^{ab,x}$	$8.0^{ab,x}$	$10.7^{bc,x}$	$15.6^{c,x}$
Hunter L		C	$47.0^{a,x}$	$50.6^{b,x}$	$51.2^{b,x}$	$51.2^{b,x}$	$52.1^{b,x}$	$51.4^{b,x}$
		H	$43.0^{a,x}$	$45.6^{b,x}$	$49.0^{c,x}$	$49.4^{c,x}$	$50.2^{c,x}$	$49.3^{c,x}$
Hunter a		C	$19.8^{b,x}$	$18.8^{ab,x}$	$18.6^{ab,x}$	$19.1^{ab,x}$	$19.2^{ab,x}$	$18.2^{a,x}$
		H	$22.2^{c,x}$	$21.3^{c,x}$	$19.5^{b,x}$	$18.7^{ab,x}$	$18.2^{a,x}$	$17.7^{a,x}$
Hunter b		C	$7.5^{a,x}$	$7.8^{a,x}$	$8.4^{ab,x}$	$8.9^{bc,x}$	$9.6^{c,y}$	$9.5^{c,y}$
		H	$6.7^{a,x}$	$8.0^{b,x}$	$7.6^{ab,x}$	$7.3^{ab,x}$	$7.1^{ab,x}$	$7.1^{ab,x}$
	storage time							
$R_{630}-R_{580}$	7		$15.3^{a,x}$	$16.5^{b,x}$	$16.5^{b,x}$	$16.5^{b,x}$	$16.3^{ab,x}$	$15.4^{a,x}$
	14		$15.0^{a,x}$	$16.0^{b,x}$	$16.3^{b,x}$	$16.8^{b,x}$	$16.3^{b,x}$	$14.4^{a,x}$
	storage x treatment							
Saturation index	7	C	$21.3^{b,x,m}$	$20.2^{ab,x,m}$	$20.7^{ab,x,m}$	$21.0^{ab,x,m}$	$21.6^{b,x,m}$	$21.1^{ab,x,m}$
	7	H	$28.8^{c,y,m}$	$28.9^{c,y,n}$	$21.3^{b,x,m}$	$20.0^{a,x,m}$	$19.3^{a,x,m}$	$19.3^{a,x,m}$
	14	C	$21.3^{c,x,m}$	$20.5^{abc,x,m}$	$20.2^{ab,x,m}$	$21.1^{bc,x,m}$	$21.4^{c,x,m}$	$20.1^{a,x,m}$
	14	H	$20.6^{b,x,n}$	$21.7^{c,x,m}$	$20.6^{b,x,m}$	$20.0^{b,x,m}$	$19.7^{ab,x,m}$	$18.8^{a,x,m}$

^{abc}Means in the same row with the same superscript letter are not different (P>0.05).

^{mn}Means of treatments within vacuum storage time in a column with some superscript are not different (P>0.05).

^{xy}Means of vacuum storage time or treatment with same superscript are not different (P>0.05).

C = chilled, H = hot boned.

Table 13 - Hue angle values of ground cutaneous truncii muscle during oxygenation.

Time (min)	Hue angle
0	2.9[a]
5	2.4[b]
10	2.3[c]
15	2.2[c]
30	2.1[ab]
60	2.0[a]

[abc]Means with same superscript letter are not different (P>0.05).

Figure 1. MetMb on the surface of ground adductor muscle chilled (AC), hot boned (AH) or after 7 (A7) and 14 (A14) days vacuum storage or of cutaneous truncii (C) during display at 3° C.

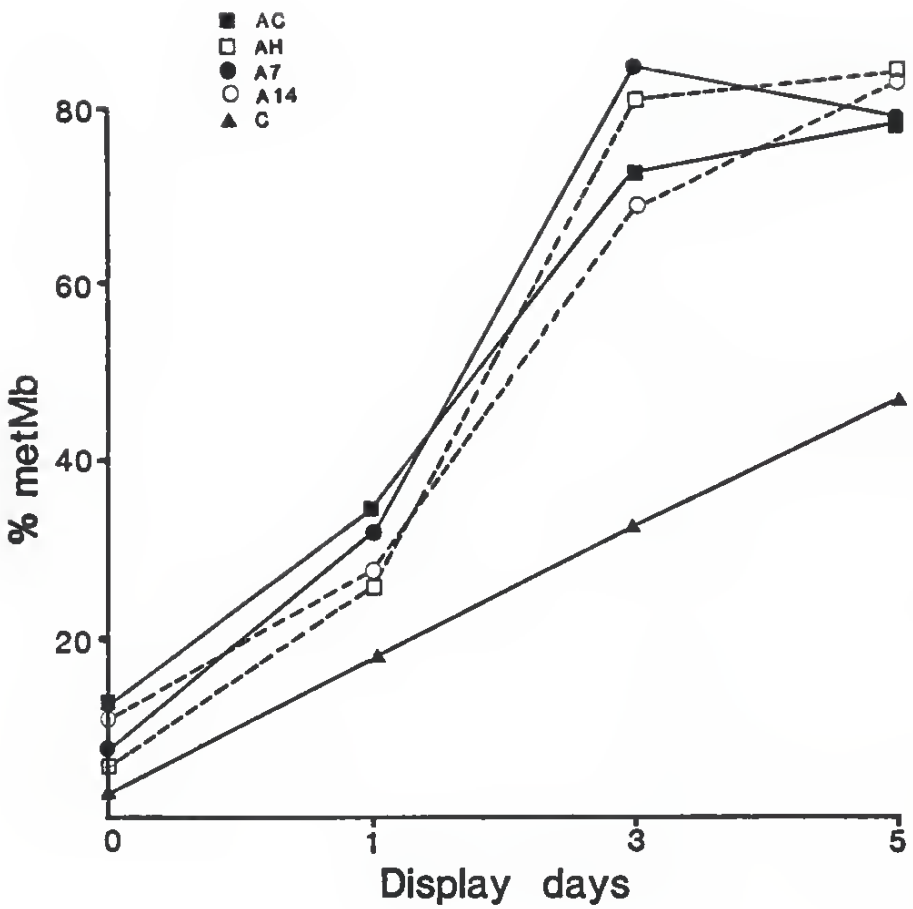

AC
AH
A7
A14
C
80
60
40
20
% metMb
0
1
3
5
Display days

Figure 2. Visual scores of ground adductor (A) and cutaneous truncii (C) for samples stored 7 or 14 days and of cutaneous truncii processed hot (CH) and chilled (CC) during display at 3° C.

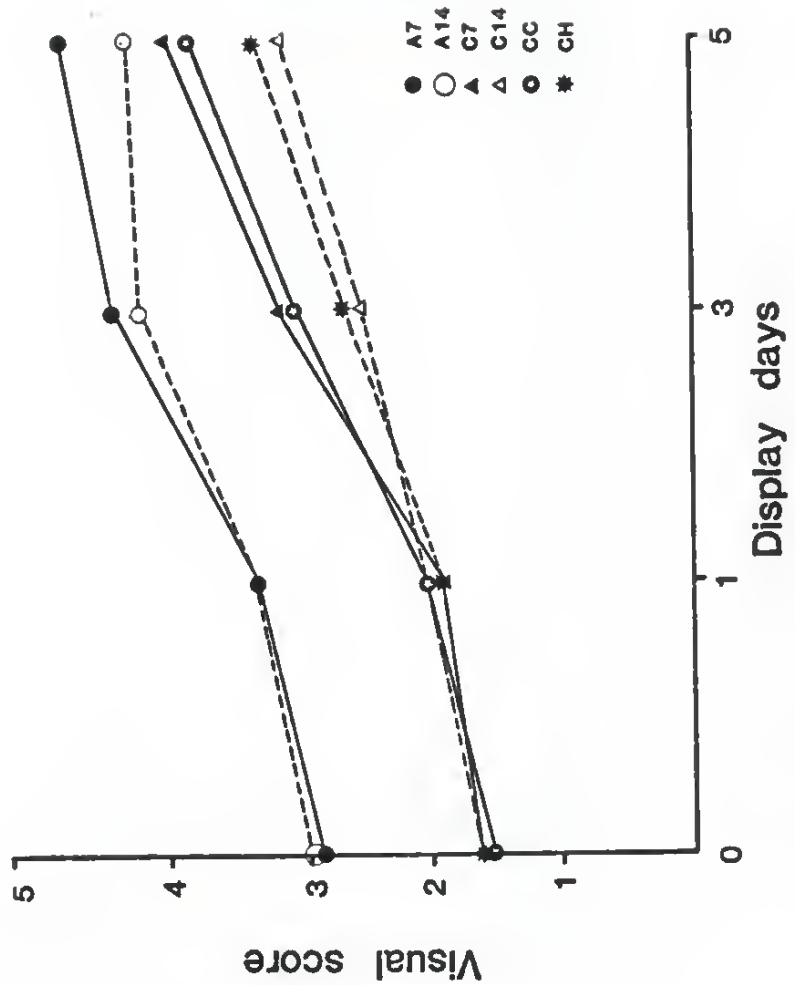

Visual score
5
4
3
2
1
0
1
3
5
Display days
A7
A14
C7
C14
CC
CH

Figure 3. MetMb (%) for ground adductor after 7 (A7) and 14 (A14) days storage and of cutaneous truncii of chilled (CC) and hot (CH) treatment during oxygenation.

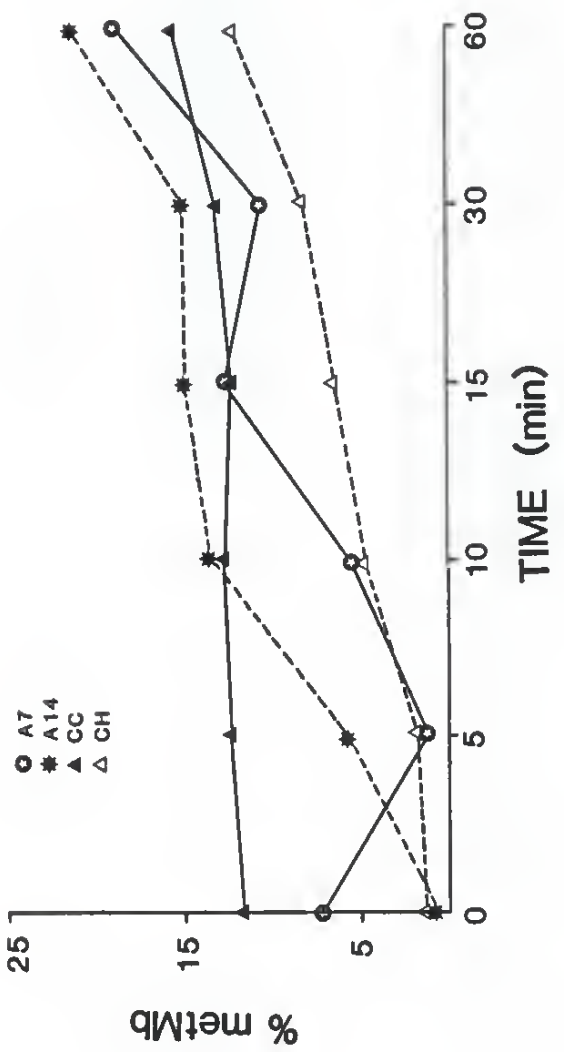
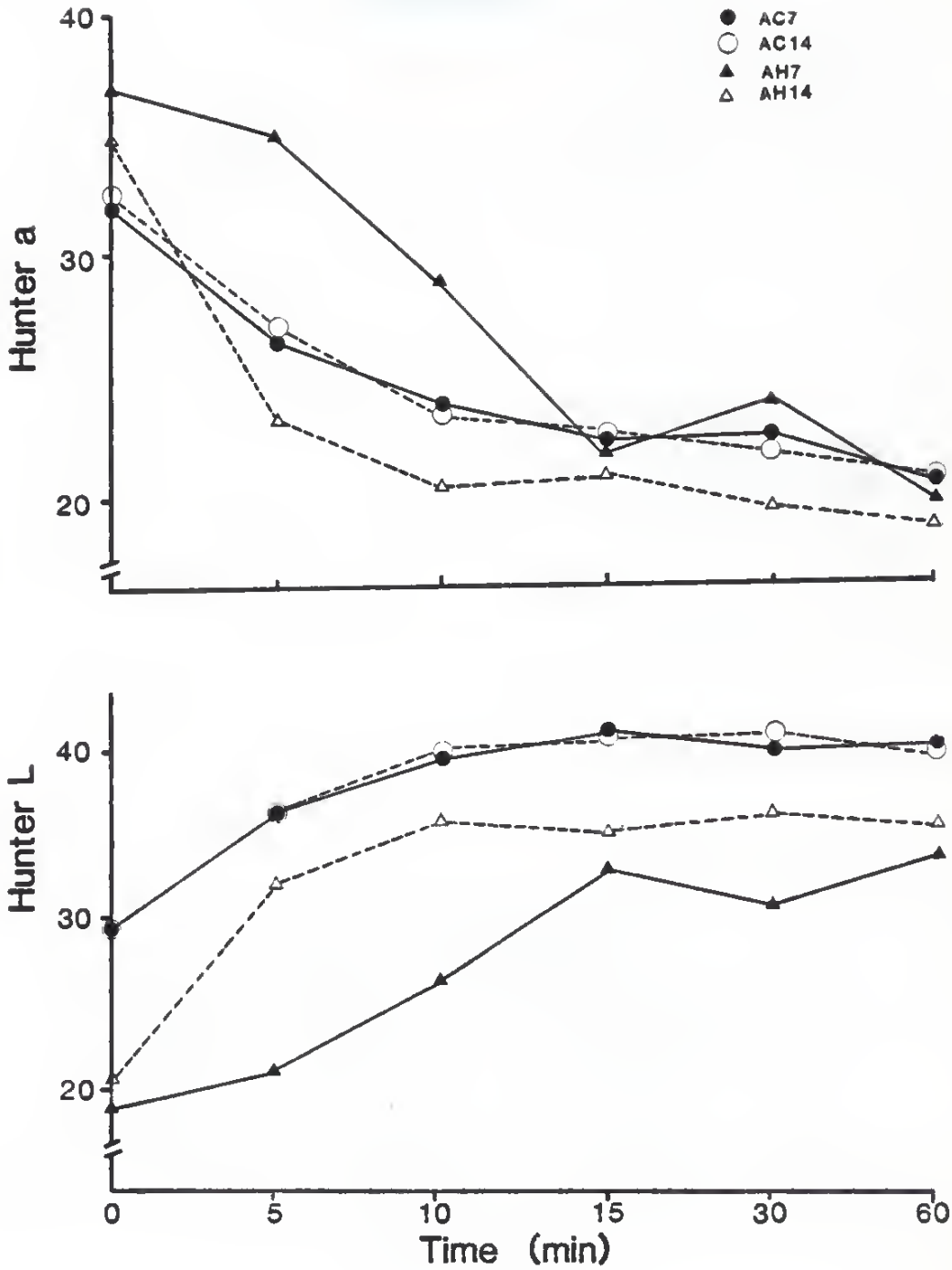

% metMb
25
15
5
0
5
10
15
30
60
TIME (min)
A7
A14
CC
CH

Figure 4. Hunter L and a values during oxygenation of ground adductor (A) muscle hot (H) and chilled (C) boned, with 7 and 14 days vacuum storage.


AC7
AC14
AH7
AH14
Hunter a
40
30
20
Hunter L
40
30
20
0
5
10
15
30
60
Time (min)

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by

DORLY PISKE

B.S. Federal University of Santa Catarina, Brazil, 1976

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Two studies evaluated the effect of oxygen exposure on ground beef color stability. First, semimenbranosus muscle (48 hr postmortem) of 3 beef carcasses was trimmed, cut and ground in a nitrogen atmosphere, compared to 48 hr exposure of muscle to air before grinding. Samples stored in vacuum 7 and 14 days, were displayed under natural fluorescent lighting at 3° C for 5 days and these color traits were measured: % metmyoglobin (metMb), difference of 630 and 580 nm reflectance (R_{630}-R_{580}), HunterLab values, saturation index, hue angle and visual score. MetMb increased faster ($p<0.05$) for samples processed in air after 14 days in vacuum, but not for those stored 7 days. Hunter L values of 14 day samples were higher ($p<0.05$) when processed in air; treatment did not affect Hunter a values. Samples stored 14 days in vacuum oxygenated better and were redder throughout display (higher R_{630}-R_{580}, lower visual score, higher Hunter L) than for 7 days storage. Samples processed in air, had very little oxyMb formation. Treatment did not affect ($p>0.05$) aerobic metmyoglobin reducing activity (MRA), although anaerobic MRA was greater for the nitrogen treatment. In the second study, a surface muscle, cutaneous truncii and a deeper muscle, adductor, of 3 beef carcasses were excised 1 hr (H) and 48 hr (C) postmortem and packaged in vacuum 7 and 14 days before evaluation of display color stability (visual score, % metMb, R_{630}-R_{580}, HunterLab, saturation index and hue angle), anaerobic MRA and pigment oxygenation. Adductor visual scores indicated a rather dark color throughout the display, but were less dark after 5 days display ($p<0.05$) with 14 days vacuum storage. Hunter L values did not change during display for samples stored 7 days, but after 14 days storage were higher from day 3 of display on ($p<0.05$). MetMb of C and H treatment was not different ($p>0.05$). R_{630}-R_{580} tended higher for H. MRA did not show consistent trends and no difference for treatment or storage. During oxygenation, C samples became lighter with decreased Hunter a over time for both 7 and 14 days storage, with no difference between

storage times ($p>0.05$). Samples stored 7 days tended toward lower lightness. Saturation index decreased over time and H had higher values than C during most of the oxygenation. MetMb formation was not different between C and H. Up to 15 min oxygenation, C showed higher $R_{630}-R_{580}$ suggesting more oxyMb than H. Cutaneous truncii display visual scores were low (bright red), with C and H not different, except at day 5 when C was higher (darker). No treatment difference was found for Hunter a, $R_{630}-R_{580}$ and saturation index. Seven days storage caused darker visual scores from day 3 of display on. Hunter a and saturation index were higher on days 3 and 5 of display for samples stored 14 days. Treatment effect of hue angle was significant for C7 ($p<0.05$) but not for C14, with no storage time effect ($p>0.05$). MRA was greatest for C7 and lowest for C14 and H7. $R_{630}-R_{580}$ decreased over time for both C and H; but slightly less for H. During oxygenation, $R_{639}-R_{580}$ changed very little for either 7 or 14 days vacuum storage samples. % metMb increased over time. No treatment effect was found ($p>0.05$) but metMb formation tended to be delayed for H samples. Hunter L was not different ($p>0.05$) between either treatment or vacuum storage time, although C tended lighter than H.