COLOR DISPLAY LIFE UNDER TWO PACKAGING SYSTEMS OF LONGISSIMUS AND SPINALIS DORSI MUSCLES FROM BULLS, ZERANOL IMPLANTED BULLS AND STEERS

by

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GENERAL INTRODUCTION

Beef production from intact males has lately received considerable interest, since bulls gain faster and more efficiently. Bull carcasses are leaner and have higher retail yields. Higher efficiency and yields translate into lower cost, and since consumers prefer leaner and lower cost beef, beef production from intact males may play a very important role in the future.

Quality characteristics of bull meat may determine consumer acceptance or rejection of bull meat. For young bull meat, tenderness, juiciness and flavor are not different than from steer meat. Color, however, has been shown to be darker for meat from bulls than from steers. Color has a direct influence upon the consumer acceptance of meat, since it is often associated with freshness, age of the animal and post-mortem treatment; and elicits a definite consumer response, i.e., buying or not buying. Realization of meat characteristics such as flavor and tenderness, is thus conditional upon apppearance of the product in the display case, which is strongly influenced by color.

Some characteristics of bull carcasses are modified by subcutaneous implanting with the growth promotant agent Zeranol, so they resemble steer carcasses in some traits. Some of these traits, as subcutaneous fat thickness, or incidence of dark cutters, may affect lean meat color.

Centralized meat fabrication and packaging has a potential for increased use. Packaging film characteristics, mainly oxygen and vapor permeabilities, greatly influence meat color, since within package atmosphere is a major factor

affecting color. Moreover, packaging systems are important in determining product display life. With increasing energy shortage and costs, any increase in product shelf life is of great economic importance, particularly for centralized packaging operations, which must ship their products to distant locations.

The purpose of this research, is to study under two different packaging systems the display behavior of two muscles from bulls, Zeranol implanted bulls and steers.

In chapter III, color display behavior of frozen longissimus dorsi and spinalis dorsi from bulls, implanted bulls and steers is reported, while chapter IV deals with the same muscles when unfrozen and vacuum packaged in high oxygen-barrier film.

Chapter V examines the simple linear correlation coefficients of spectrophotometric meat color measurements with visual color scores of these two muscles.

CHAPTER II

GENERAL REVIEW OF THE LITERATURE

MEAT PRODUCTION FROM INTACT MALES

Performance

In recent years meat production from intact male cattle has received considerable attention. Abundant research has been done on bull performance as compared with steers, showing a definite advantage in their use for red meat production. Extensive reviews of this topic were published by Seideman et al. (1982) and Field (1971).

Growth performance of bulls and steers has been studied at different ages. Glimp et al. (1971), Bayley et al. (1966), and Brown et al. (1962), found no difference in pre-weaning performance of intact vs castrated beef males.

Contradictory reports were published considering the effect of age of castration. Champagne et al. (1969) found no difference in feedlot performance of steers that had been castrated at birth, two, five and seven mo; Klostermann et al. (1954) found no difference between castration at one or seven mo, and Glimp et al. (1971) found that steers castrated at birth grew faster than those castrated at weaning. Klostermann (1954) reported advantages in feedlot bulls vs steers of both castration ages. Bulls gained significantly faster, and required less feed per unit of gain . Similar results were found by Brown (1962), Aitken

et al. (1963), and Nichols et al. (1964). Hedrick et al. (1969), found bulls superior to steers in liveweight gain and feed conversion. In a study involving two different feed intake levels, Marlowe and Gaines (1958) reported a 5% advantage in growth for bull calves compared to steer calves. When adjusted to a weaning age of 210 d liveweight, bulls were 16 lb heavier than steers at the same age. This diffference was slightly larger when creep fed calves were considered.

No difference in liveweight gains between bulls and steers is expected until sexual development begins. Bayley et al. (1966) found a similar preweaning growth rate between bulls and steers. In the feedlot, however, bulls grew more rapidly and were more efficient in feed conversion than steers. In an experiment conducted with grazing cattle between 58 and 170 d of age, unilateral castrates and intact males grew slightly faster than bilateral castrates. With the onset of puberty, and its consequent libido increase, the difference in growth rate was narrowed (Watson, 1969). These results, as well as those of Bayley et al. (1966), apparently show a greater difference between bulls and steers when the animals are allowed a high energy intake. This difference is supported by Turton (1962), who suggested that under grazing conditions, full castrates had a higher growth rate than intact males or unilateral castrates. This observation is supported by Tylececk (1958), as quoted by Seideman (1982). Martin et al. (1978), showed that protein intake is the most important factor in determining the advantage of bulls since there were no differences between bull and steer growth at low protein intake.

Effect of castration on carcass characteristics and cutability

Allen (1982) summarized various studies comparing carcass composition of bulls and steers (Table 1). Jacobs et al. (1977a) examined carcass characteristics of Hereford bulls and steers, and found bull carcasses had larger rib eye areas, less trimmable fat and higher retail yields than steer carcasses. Carcasses from steers, however, had higher quality grades than from bulls. Bull carcasses yielded 16% more edible meat than steers, had 58% less crude fat, and 23% more crude protein. Cutting losses were less for bulls than for steers, resulting in higher retail yield for bull carcasses. In a study involving Angus bulls and steers, (Arthaud et al., 1969), bull carcasses were heavier, and yielded more retail product but had a lower grade than steer carcasses.

Arthaud et al. (1977) noted a consistent tendency of bull carcasses to exhibit more skeletal and lean maturity than steers at the same chronological age.

Carcass composition differences are related to energy intake level (Arthaud, 1977). Percentage fat in rib cuts from bulls fed high energy level was only slightly higher than from low energy fed bulls. For steers, however, the fat percentage in rib cuts was much higher in the high energy level group, than in the low energy fed steers.

Bulls, having less fat, were found to have a lower dressing percentage than steers (Field, 1971). Nichols (1964) found a slightly lower dressing percentage for bulls, mostly due to their heavier hides and less fat carcasses. The percentage of desired cuts, however, was higher for bulls. These results agree with those of Klostermann (1954).

Meat characteristics

Arthaud et al. (1977) found steer carcasses to have more marbling and firmer meat texture than those from bulls. Tenderness has been reported as slightly less in bulls than steers (Field, 1971). Boccard et al. (1979) found that collagen content was higher in longissimus dorsi, semimembranosus, semitendinosus, pectoralis profundus and triceps brachii muscles from bulls than steers, with collagen solubility decreasing with age (especially after puberty). Hedrick et al.(1969) found bull meat tenderness comparable to steer meat when age was below 16 mo. Steaks from mature bulls were found less tender than those from steers of comparable age (Arthaud et al., 1977). Jacobs et al.,(1977b) found Warner Bratzler shear values for bulls higher than those for steers, both at 18 to 19 mo of age.

Bull meat has less fat, and more moisture, protein and ash, than steer meat (Arthaud et al., 1969; Arthaud et al., 1977). Jacobs et al., (1977a), studying chemical compositon, found bull meat to have higher moisture content, less ether extract and more crude protein than steer meat. Longissimus dorsi from steer carcasses had a higher ether extract than from bulls (Bayley, 1966). This occurs especially when the animals are fed high energy diets. Cross and Allen (1982) reported less intramuscular fat and lower USDA quality grades for bull carcasses.

Less subcutaneous fat could make some bull carcass muscles more susceptible to cold shortening, thus being responsible for part of their tenderness disadvantage compared with steers.

Field (1971) summarized information from seven studies regarding palatability characteristics of bulls and steers. Steers ranked higher than bulls in tenderness, juiciness and flavor. Tenderness differences were further affected by age, while flavor and juiciness were not. Hood and Allen (1971) quoted by Seideman et al. (1982), reported no significant sex difference in boiled beef flavor.

Smith (1982), reviewed the information concluding lower quality of bull and bullock meat. He considers the most important factors affecting bull meat quality when compared with steer meat to be age because large differences in toughness result from relatively small increases in age, as from 15 to 18 mo in Hereford bullocks (Carpenter et al., 1967); breed since Brahman bullocks are "most likely to be tough" (Smith et al., 1982); "masculinity", with tenderness being less in "very masculine" than in "slightly masculine" bullocks (Riley et al., 1982a); electrical stimulation, with improved tenderness of E.S. vs. non E.S. bullock sides (Riley et al, 1982a); and subcutaneous fat thickness because U.S.D.A. Good and U.S.D.A. Standard bullocks with more than .30 in. or more of external fat at the 12th-13th rib interface produced steaks as tender as those from U.S.D.A. Choice Steers (Riley et al., 1982b). These two last factors, seem to explain the toughness of bull meat on a cold shortening susceptibility basis.

Muscle color differences in meat from bulls and steers

Color is a very important factor in determining consumer acceptance of bull meat. Color was rated by the consumers as the second most important factor in selecting retail beef (Jacobs et al. 1977b).

There is considerable evidence in the literature finding bull meat darker in color than steer meat (Seideman et al., 1982). Field (1971) quotes four references finding bull meat darker in color than steer meat. Arthaud et al. (1977) found steer meat to have a more desirable bright red color. In another study (Arthaud et al., 1969), lean color was scored darker for bulls regardless of their dietary energy intake. Jacobs et al.(1977b) found bulls to have a slightly darker meat than steers. Copious evidence of darker bull meat is reviewed by Cross and Allen (1982). Smith (1982) adds further references supporting this difference.

No significant differences were found in myoglobin content of bull and steer longissimus dorsi (Arthaud et al., 1977). Boccard (1979) studying longissimus dorsi, semimembranosus, semitendinosus, pectoralis profundus and triceps brachii muscles, and Purser (1982), for top round, bottom round and rib eye cuts, found no differences in total pigment content between bulls and steers. These results are in agreement with those of Watson (1969), who found no appreciable differences in myoglobin concentration between bull and steer longissimus dorsi when the animals were 10 mo old. However, when 22 mo old unilateral castrates were compared with bilateral castrates , longissimus dorsi from unilateral castrates (measured by subjective evaluation) was found darker.

Although he concludes otherwise, Purser (1982) shows a significant difference in trained panel color scores between bulls (darker) and steers. This difference was present in top round and ribeye cuts, but not in bottom round. Overall desirability of appearance was also rated higher for steers than for bulls. His objective measurement results, also show differences in higher "L" and "b" values for steer ribeye muscle over bulls at d 0 and 3 of display, as well as an increase in "a" values at day 3 of display for steaks from top rounds. Higher "b" values in both steer ribeye and top round steaks, "suggest an increased metmyoglobin formation" at the initial phase of display.

Field (1971) suggested a higher incidence of "dark cutters" in bulls as being responsible for their darker lean color. This topic has been further reviewed by Smith (1982), and Cross and Allen (1982). Bull muscle is frequently found to have higher pH values than steer muscle. This factor is commonly associated with problems during transport (Payne, 1969)

Bull meat samples of normal pH had normal visual color scores and objective color measurements (lightness, hue and saturation) in the normal range for steers (Mc Dougall & Rhodes, 1972). When darkness occured, it was mostly in high pH samples.

Purser (1982) found higher mean pH values for bull top round and rib eye muscles, when compared with steers. No differences were found in bottom rounds. Coincidently, this was the cut in which no differences in color were observed, either by visual or instrumental color measurement.

Effect of Ralgro implanting on bulls

Greathouse (1982) reviewed the effect of Ralgro on the performance, carcass composition, yield, maturity and quality factors of beef cattle. In his study, bulls implanted with Ralgro from birth to slaughter gained more efficiently than non-implanted bulls. Carcasses from implanted bulls were more "physiologically mature", and had more tender longissimus muscle.

Unruh et al. (1983), found increased hip height at weaning and slaughter for bulls than for steers; zeranol implanted bulls had an intermediate height. The same pattern was followed by masculinity traits, carcass weights and longissimus area, while fat thickness was greater for steers than for bulls, with implanted bulls again intermediate.

Gray et al. (1983) compared bulls, implanted bulls and implanted steers, and found bulls to have intermediate carcass traits between non-implanted bulls and steers. Time and duration of implanting determined the magnitude of the observed effects. Johnson et al. (1983) found no effect of Ralgro implants on palatability traits of bulls. Patterson et al. (1983) found increased subcutaneous fat in Ralgro implanted Gelbvieh bulls.

MEAT PIGMENTS

Fresh meat color is probably the most important appearance characteristic affecting its saleability, with the exception of cost and package size. Color is used by the consumer as a means of estimating age and past history of the product. Hiner (1954) states color elicits both a real response (quality estimation), and a psychological response, (acceptance or rejection). Although good appearance is not necessarily an estimator of good texture and flavor (the attributes that really count at mealtime), the shopper associates bright red color in display with superior eating quality (Giddings, 1977). Thus, color can determine the chance other meat attributes have of being experienced.

The color of fresh meat is due mainly to its major pigment, myoglobin, and the chemical form, mostly degree of oxidation of this pigment. Hemoglobin, plays a secondary role in meat color, adding up to 20 to 30 % of the total pigment in some muscles (Fox, 1966). The difference in total pigment content of some muscles is due to differences in their hemoglobin content, more than myoglobin.

Myoglobin concentration in muscle is dependent on animal species, and principally on the individual muscle considered. Hunt and Hedrick (1977) found myoglobin content of beef muscle ranging from 1.95 mg/g of tissue, to 4.11 mg/g. Mac Dougall (1977) reported differences in pigment concentration in beef muscle to range from 3.6 mg/g ("normal") to 6.3 mg/g ("high pigment").

Rickansrud and Henrickson (1967) studied myoglobin concentration in four bovine muscles and reported myoglobin content ranging from 1.99 mg/g tissue for semitendinosus (ST), to 3.64 mg/g for the biceps femoris (BF). This difference grows even larger if pigment concentration is reported on a percentage of dry, fat free matter, the range then being from 8.72 (ST) to 15.70 (BF). They found hemoglobin content to be a significant factor in visual color differences between muscles. These data differ from those from Hunt and Hedrick (1977), the difference being presumably accounted for differences in holding time of the carcasses (Livingston and Brown, 1981).

Tuma (1971) compared beef longissimus dorsi (LD) and spinalis dorsi (SD), obtaining pigment contents in "lighter" colored rib steaks of 4.38 for LD, and 5.24 for SD. For "darker" rib steaks, (visual score of 3 or higher), total pigment content was 4.69 for LD and 7.51 for SD.

Myoglobin content closely parallels the fiber type composition of a given muscle or set of muscles, being higher in red than in white muscle (Cassens and Cooper, 1971).

Sex affects pigment concentration of muscle, bulls having somewhat higher total pigment than steers at the same age (Mac Dougall and Rhodes, 1972).

Myoglobin is a monomeric, globular heme protein, with a molecular weight of aproximately 18,000; localized in the muscle sarcoplasm. Hemoglobin is a tetrameric globular heme protein (four hemes per molecule), localized in the erythrocytes (Livingston and Brown, 1981). An extensive review of myoglobin composition and properties has been done by Giddings (1977), and more recently by Livingston and Brown (1981).

Essentially, the myoglobin molecule is composed of a protein portion (globin), folded in a globular shape. The heme group, consisting in an iron atom and a porphyrin ring, is located in a cleft of the globular molecule.

The chemical status of the iron nucleus in the heme ring is responsible for the principal changes observed in fresh meat color. The native reduced myoglobin is the pigment form with the iron in the ferrous form, and normally occurs under very low oxygen partial pressure conditions, as occur in living muscle, or in muscle deep layers where intracellular oxygen tensions are below .1 mm Hg. This is the purple red form naturally found in fresh meat immediately after cutting (Livingston and Brown, 1981).

On exposure to the air, the iron's 6th ligand position is oxygenated, thus resulting in oxymyoglobin, the bright red pigment form that imparts the characteristic red meat color.

Oxidation of the iron by one or two electron oxidants, changes the pigment form to metmyoglobin , with its iron in the ferric form. Metmyoglobin has an undesirable brown color which becomes detectable when it adds up to 40% (Harrison et al., 1980; Greene,1971) or 60 % (Brooks,1938; Van den Oord and Wesdorp, 1971a) of the total pigment. Iron in myoglobin exists either as Fe^{++} (Mb or OMb), or as Fe^{+++} (MMb).

Livingston and Brown (1981) summarized the main factors determining the pigment forms of fresh meat as: 1. oxygen tension; 2. temperature; 3. pH; 4. salt concentration, 5. reductant levels, and 6. lighting characteristics. Schweigert (1956) concluded that although oxygen is required for formation of oxymyoglobin from reduced myoglobin, a prolonged exposure to oxygen results in Mb^+ formation, with oxidation of the iron to the ferric state.

George and Stratmann (1952) reported that maximum rate of oxidation of myoglobin to metmyoglobin occurs at partial O_2 pressures between 1 and 1.4 mm. of Hg. This maximum, however, could be shifted by altering pH and temperature (Solberg, 1968).

Centralized prepackaging makes the effect of oxygen partial pressure on myoglobin form an increasingly important phenomenon; first, because a higher percentage of meat surfaces are exposed to the atmosphere, and second, due to the effect of packaging films in modifying this atmosphere (Lawrie, 1983).

The depth of the oxygen penetration into a cut meat surface is dependant on the oxygen partial pressure, temperature (O_2 penetration increases at low temperature) and the rate of oxygen consumption by residual enzyme activity (Mac Dougall, 1977). Myoglobin oxidation rate is maximal at a low pO_2, and when residual enzyme activity is lost, metmyoglobin is formed as a band near the limit of the oxygen penetration zone.

MEAT COLOR MEASUREMENT

Hunt (1980), recently reviewed meat color measurement systems. The method of choice in each case, will depend on the objectives of the research.

They can be classified in the following categories: 1. evaluating sample color; 2. grading; 3. studying consumer response; 4. developing marketing specifications; and 5. detecting deteriorative color changes due to processing or display procedures.

We can basically consider two approaches when measuring meat color. One is a physical description of the perceived color, that is, a comparison against a given standard or set of variables (Munsell, Hunter, CIE - tristimulus). The Hunter (Gardner) system has been widely used for meat color measurements, its readings going from +a for redness to -a for greenness; -b for blueness and +b for yellowness, while the degree of lightness is given by the L vertical scale (Francis, 1971).

A second approach would be a study of the relative concentration of the different pigment forms, such as in spectrophotometric techniques. Transmission and absorbance spectrophotometry, although useful methods for total pigment evaluation, distort the pigment profile too much to be used in relative pigment form determinations. This distortion is mostly due to changes in the more unstable reduced form to oxymyoglobin and metmyoglobin (Dean and Ball, 1960). Reduced myoglobin is almost completely oxygenated during extraction (Krzywicky, 1982). However, the proportion of reduced to oxidized fractions (Fe^{+++} to Fe^{++}) appears to remain constant for a short time.

Hunt (1980) summarized the available information about the different wavelengths used for measuring meat color. The use of ratios or differences of reflectance at two selected wavelengths, helps to correct for differences in pigment concentrations between samples, as well as for texture or marbling differences. Particularly interesting are measurements of %R474 nm/%R525 nm

as an estimator of reduced myoglobin, and of %R572 nm/%R525 nm, as an estimator of metmyoglobin (Snyder, 1965; Stewart et al.,1965a; Ledward, 1970). The ratio of percent reflectance at 630 nm / 525 nm, was used as an estimator of metmyoglobin (MMB) accumulation (Claus, 1982), while %R 630 nm minus %R 580 nm was suggested as an estimator of "redness", or proportion of reduced myoglobin (MB) and oxymyoglobin (OMB) accumulation (Strange et al.,1974; Harrison et al.,1980; Sleper, 1982). Correlations for this ratio with visual score were aproximately 0.80.

More recently, Hunt (1982) suggested reflectance at 610 nm as being a useful wavelength for meat pigment estimations. Reflectance at this particular wavelength is isobestic for MB and MMB, coincidently with a maximum for OMB. Thus, the %R at 610 nm - %R at 525 nm, might be used a an estimator of OMb, as well as %R 610 - %R 580 and %R610 - %R572.

Translation of reflectance data into K/S values, corrects for some intrinsic optical properties of the sample (Francis and Clydesdale, 1965; Hunt, 1980), linearizing the relationship between reflectance and pigment concentration. Pigment proportion estimates from reflectance data, are improved by conversion to K/S values. To study the relationship with visual scores, however, Harrison et al.(1980) did not find any improvement in the correlation coefficients to visual color scores when data were converted to K/S values.

Krzywicky (1982), recently proposed using reflex attenuance (log 1/R; Shibata, 1966) as an estimator of muscle pigment concentration, in order to compensate for the muscle structure optical properties (i.e., the meat absorbtion of light). To account for texture effects, he suggested using the reflex

attenuance at 730 nm as a standard, that is, as the muscle completely deprived of pigments. The concept of reflex attenuance is not new, however. Naughton et al.(1958), and Dean and Ball (1960), quoting Wodicka (1956), report using the log of the reciprocal of reflectance (absorbancy) for meat color measurements, although they did not estimate pigment forms .

FRESH MEAT COLOR

The color, and with it the acceptability of a fresh meat product, will be affected by total pigment concentration, along with certain properties affecting light scattering (Livingston and Brown, 1981). Since myoglobin very rarely occurs in a single pigment form, the relative concentration of its different forms will affect and characterize the color of the product.

When the purple red form, MB, is exposed to the air, the pigment is oxygenated to the bright cherry red form, OMB. This form, however, is relatively unstable, and tends to change to the unpleasant brown MMB form (Mac Dougall, 1977).

Hence, the relative proportion of different forms of pigment at or near the surface of the product will depend on the availability of oxygen above that surface (Brooks, 1938), and its penetration into the tissue. This factor, is itself dependant upon oxygen uptake by the tissue and the rate of oxygen diffusion into it (Lawrie, 1958).

The brightness of the red color, depends on the depth of oxygen penetration and oxygenation of myoglobin. Myoglobin oxidation is maximal at

pO_2 of 1 to 1.4 mm of Hg (George and Stratmann, 1952). Thus, in oxygenated fresh meat, MMB is first formed at the interface between the oxygenated layer and the reduced myoglobin layer; that is, near the limit of the oxygen penetration (Mac Dougall, 1982). The difference in appearance of meat samples, would be due to the thickness of the oxymyoglobin layer at the surface (Krzywicky, 1979).

Oxygen penetration into a muscle is affected by oxygen diffusion into meat and the rate of oxygen uptake of the tissue (Bendall 1972). This uptake is highly dependent on the pH decline post-mortem and its effect on natural metabolic pathways by means of substrate depletion and enzyme denaturation.

The color of beef at "normal pigment concentrations" and with a MMb fraction below 30%, is highly affected by the optical characteristics of the meat surface (Krzywicky, 1979). This can possibly account for some muscle differences not explained by pigment profile differences. Mac Dougall (1982), illustrates the importance of light scatter in fresh meat color. Small changes of scatter coefficients bring about very important changes in visual lightness L at constant pigment concentrations.

DISCOLORATION OF BEEF SURFACES

The bright red color of OMB meat surfaces is unstable, and deteriorates to the less attractive brown color of metmyoglobin. This phenomenon is referred of as discoloration. Hood and Riordan (1973), evaluated the consumer response to discolorated meat, finding a linear inverse relationship between the proportion of total sales of discolored beef and its metmyoglobin content. When

30% or more metmyoglobin was present in the discolored batch, the ratio of sales of discolored vs. normal meat was 1 to 2.

When meat is stored in air, the oxymyoglobin layer is about 2 to 4 mm in thickness (Brooks, 1929). As storage time increases, the oxidation of myoglobin to metmyoglobin continues, and the metmyoglobin band thickens. The oxymyoglobin layer is now too narrow to continue masking the unattractive adjacent layer, and brown discolored areas appear in the surface (Taylor and Mac Dougall, 1973). The increase of metmyoglobin in the surface layer is shown by a gradual loss in reflectance at 630 nm, a yellower hue and decreasing saturation (S). Mac Dougall (1977) considers S values over 20 as bright red; 18 as dull red; 14 as distinctly brown and values under 12 are brown to grey-greenish brown.

MAJOR FACTORS THAT AFFECT DISCOLORATION AND MEAT COLOR STABILITY

The most important factors affecting meat stability and meat color discoloration, besides individual muscle characteristics (Ledward, 1971; Hood, 1980) are **temperature, gaseous environment, oxygen consumption rate** and **reducing capacity of the meat** (Mac Dougall, 1982).

Temperature: Mac Dougall and Taylor (1975) found the rate of meat discoloration in display cases to be doubled by an increase in the display temperature of 3 C. O'Keefe and Hood (1980 b), found increased temperature as being the most important factor, apart from age post-mortem, in affecting muscle color stability. Brown and Mebine (1969) found that the OMb oxidation

rate decreased approximately 40 fold when temperature was decreased from 22 C to -2 C. Not only temperature by itself, but temperature fluctuation affects color stability. Literature reviewed by Kropf (1980) agreed in observing an increase of meat surface discoloration with an increase in temperature.

Temperature, however, does not apparently affect the reverse pigment transformation. Griffin et al. (1982) found no significant difference in the reduced myoglobin purplish red color development in round steaks stored at $2^{o}C$ or $7^{o}C$, but age post-mortem was a significant factor.

Apparently, muscle characteristics are also important in this temperature effect. O'Keefe and Hood (1980a), found this shelf life reduction effect as being proportionally the same for the different muscles studied (psoas major, gluteus medius and semimembranosus) when these were stored in air. When anaerobic storage was used, however, the effect of temperature on subsequent aerobic shelf life was related to intrinsic muscle characteristics, muscles with less color stability (e.g. psoas major) being more susceptible to the effect of increased temperatures.

The main factor responsible for increased color deterioration in some muscles is an increased rate of myoglobin oxidative reactions.

The temperature effect is apparently reversed when temperature falls below the freezing point. Brown and Dolev (1963) conclude that the faster oxidation rate of myoglobin at subfreezing temperatures is due to physical arrangement of the myoglobin and oxygen molecules. Probably, a solute concentration effect also occurs in frozen meat (Fennema, 1973). Oxidation of frozen meat is different than in normal fresh meat. In this condition, the most

important factor is the photooxidation phenomenon, the meat oxidizing from the surface inwards (Mac Dougall, 1982).

Gaseous environment The effect of gaseous atmosphere on meat pigments was shown by Brooks (1931). George and Stratmann (1952) studied the relationship between myoglobin oxidation and partial oxygen pressure. Holland (1980) reviewed controlled atmosphere packaging techniques, and Kropf (1980) reviewed the effect of the gaseous environment on the color of fresh meat in display. The most important gases used in modified atmosphere packaging are nitrogen, carbon monoxide,carbon dioxide, and oxygen. Nitrogen has generally been found ineffective in modifying display color. CO_2 has been extensively used as a method of ensuring longer shelf life of red meats. Concentrations of CO_2 over 25%, however, have a deletereous effect on meat color. Ledward (1970) found no effect of CO_2 atmosphere on color, provided that the O_2 content of the atmosphere was kept above 5%. In another study, Seideman et al. (1979) found no differences in oxymyoglobin content under different atmospheric compositions (100% O_2, 100% CO_2 and 100% N_2) during 28 days of storage.

O'Keefe and Hood (1980a) studied shelf life of meat after storage in anoxic N_2 and CO_2 atmospheres, and found less discoloration during display of meat stored in the presence of oxygen. This decrease in shelf life, was similar in a percent basis for muscles of different color stability, although the absolute decrease was more important for muscles with a long display life.

Seideman et al. (1980) found vacuum packaging storage gave better appearance to subprimal and reformed subprimal cuts than modified atmospheres with 20% CO_2 - 80% N_2 , and 40% CO_2 - 60% N_2.

Samples stored in oxygen show a deeper oxygenated layer and look brighter. Shelf life of steaks cut from meat stored in 100% O_2 is shorter than when the steaks come from vacuum packaged meat or from a 20% O_2 - 80% CO_2 atmosphere (Seideman et al., 1979), however steaks from the 20%O_2-20%CO_2 treatment had a better overall appearance, less discoloration , and apparently (no statistical analysis) less MMB.

Gaseous environment within meat packages can be controlled by the packaging film used. Besides its own optical characteristics, which might affect color appraisal, film gas and moisture permeability characteristics have a decisive effect in the color of meat. Pierson et al. (1970) found that oxymyoglobin of anaerobically packaged beef was completely oxidized to metmyoglobin after 5 days of storage, while reduced myoglobin in anaerobic packaging remained stable during 15 days of storage at 38 F. Kropf (1980) reviewed the importance of different packaging materials on the color of fresh meat. Physical characteristics of the film, such as glossiness, mateness, opaqueness, translucence and degree of wrinkling affect color appraisal. Color itself, is dependant on packaging film gas and moisture permeability, gas permeability determining the package atmospheric composition, and moisture permeability affecting color through surface dessication. A film permeability of approximately 5000 $cm^3 \cdot m^2 \cdot day \cdot atm.$, has been deemed necessary to keep the fresh bloomed bright red color (Sacharow, 1974; Landrock and Wallace, 1955 and Taylor, 1982).

Claus (1982) found greater color stability and more color uniformity in steaks packaged in oxygen impermeable material (Surlyn-Saran) vs. PVC packaged beef. In order to keep adequate color stability in meat packaged in

the deoxymyoglobin status, an extremely low oxygen permeability is desired. For pork, Terlizzi (1982) considers an adequate and economical permeability to be about 35$cm^3$100 in^2·24 hrs·25 C·atm. Griffin et al. (1982), found more color stability and brighter lean color when strip loin steaks were packaged in a high oxygen barrier film (10 $cm^3 \cdot m^2$·24 hr·22.8 C @ 0% RH) vs. medium oxygen barriers (30 $cm^3 \cdot m^2$·24 h·22.8 C @ 0% RH).

Degree of vacuum can affect surface discoloration. Seideman et al. (1976) found less surface discoloration for high vs. low vacuum packaged steaks (visual appraisal of vacuum) during 21 days of storage.

Oxygen consumption The ultimate thickness of the oxygenated red layer, will depend on the balance between the oxygen partial pressure at the surface, which will determine the force driving oxygen inwards, and tissue respiration, which scavenges available oxygen. Factors that depress oxygen consumption, such as low temperatures, increase oxygen partial pressure, and will enhance deeper penetration of the gas, thus promoting a deeper layer of oxygenated tissue. Myoglobin is responsible for the oxygen transport to, and CO_2 transport from the tissue (Taylor, 1982).

Solberg (1970) illustrated the oxygen uptake rate by respiring muscle. Aging will decrease oxygen uptake, thus increasing the depth of O_2 penetration (Brooks, 1929). Bendall and Taylor (1972) found muscle oxygen consumption rate after storage in chilling conditions to decay exponentially with storage time increases. In another study, the rate of oxygen penetration over a 7 d storage period in air was related to the age of the sample when exposure began (O'Keefe and Hood, 1982). These results support those of Atkinson et al.(1969). Atkinson and Follet (1973) found a steep decrease of oxygen uptake,

from 170 µl O_2·2h·g of tissue, to less than 1/3 of that value after 48 h of storage. Oxygen uptake kept decreasing slowly, to a value of 25 µl·2h·g by the 6th day. This decay of the oxygen consumption rate (OCR) is due to damage to the mitochondrial function at low pH, and is inversely related to the ultimate muscle pH. De Vore and Solberg (1974) also reported a decrease in oxygen uptake from 4.4 µl/cm^2 to 2.2 µl/cm^2 after 10 h.

OCR increases with temperature. Brooks (1929) found the depth of oxygen penetration in muscle at 0 C to be 2.2 mm; at 4 C 1.6 mm, and at 15 C 0.9 mm. Bendall and Taylor (1972), found oxygen penetration depths at the same temperatures, to be 2.2, 1.83 and 1.03 mm. This decrease in OCR at low temperatures is confirmed by Atkinson et al. (1969), Atkinson and Follet (1973), and De Vore and Solberg (1974). Increases in pH were also related to increasing OCR in post-rigor muscle (Bendall, 1972; Urbin and Wilson, 1961).

Increasing the rate of pH decline as promoted by electrical stimulation (ES), causes an important decrease in OCR, thus enhancing oxygen penetration. This can be speculated as one cause of increased "blooming", or more redness of ES meat (Bendall, 1980; Ashgar and Hendrickson, 1982). This view is supported by Tang and Henrickson (1980), who found an increase in oxymyoglobin content 4 h post-mortem after ES. Sleper et al. (1983), however, found no differences in % pigment concentration of any myoglobin chemical state at the surface of longisssimus dorsi from ES vs control muscles sampled 2 h post-mortem. In samples measured at 5 and 30 m, ES had less reduced Mb than control. When samples were bloomed 6 d post mortem, however, ES samples had lower % MB, and higher % OMB and MMB. The wide range of results found in the literature regarding the effect of electrical stimulation on lean meat color, can perhaps

be explained by the fact that the color evaluation has been done at different times post mortem. At 24 h post mortem, and with a similar rate of pH decline, OCR should be similar.

Muscle differences in oxygen uptake are important when studying oxygen diffusion into meat, with its consequent conversion of myoglobin to oxymyoglobin. O'Keefe and Hood (1982) found psoas major to have a higher OCR than longissimus dorsi, as well as a more rapid decrease in OCR in the pre-rigor period. Psoas major muscle ended with a relatively high OCR of 55 $\mu l \cdot O_2 \cdot 90$ min·g of tissue. For longissimus, OCR was reported initially lower, and decrease during storage was more gradual, attaining a relatively low final OCR of 33 μl $O_2 \cdot 90$min·g. Griffin et al. (1982) found adverse effects of 6 or 9 vs 3 d of vacuum storage for semitendinosus lean color during the first two days of display. This behavior, however, was reversed for semimembranosus, whose lean color was improved by the longer storage periods.

Myoglobin reducing capacity of meat. Dean and Ball (1960) reported a certain degree of reduction of metmyoglobin to reduced myoglobin during aerobic storage of meat. Myoglobin reduction is important to hold muscle foods under optimal conditions (Livingston and Brown, 1981). Zimmermann and Snyder (1969) illustrated metmyoglobin reducing activity (MRA) in intact beef samples.

Metmyoglobin reduction is mediated through NAD under anoxic conditions (Watts et al., 1966; Stewart et al., 1965b). Giddings (1977) and Livingston and Brown (1981) extensively reviewed the intimate mechanism of metmyoglobin reduction, concluding that the process is enzymatic in nature, and resides in the

mitochondria. Hagler et al. (1979) identified an NAD dependent reductase in bovine heart muscle.

There is a high negative correlation between metmyoglobin formation and MRA under aerobic conditions (Ledward, 1972).

The extent to which muscles use respiratory or glycolytic pathways in vivo, will determine the pattern of surviving enzymatic activity post mortem (Lawrie, 1983). Muscles of poorer stability, like psoas major, gluteus medius or semimembranosus, show a lower MRA than more color stable muscles, like longissimus dorsi. No correlation has been found, however, between MRA and discoloration rate. Ledward (1972) attributes differences in muscle discoloration to differences in the aerobic system naturally present in beef.

MRA is decreased during storage of meats, due to factors such as pH drop; depletion and/or degradation of substrates and cofactors; and damage to mitochondria (Giddings, 1974). This pH effect is also suppported by Cutaia and Ordal (1964), Stewart et al. (1965b), and Cheah (1971).

Temperature also has been reported as having an important effect on MRA. Stewart et al. (1965b), working on ground beef, found little MRA below 15 C, with a high increase with the rise of temperature up to 35 C. Ledward (1977), however, found MRA in muscles kept at 1 C. Mincing destroys the reducing capacity of meat, while partial mincing caused non-homogeneous browning.

Accelerated discoloration of aged meat (O'Keefe and Hood, 1980a), could be due to formed metmyoglobin no longer being reduced (Mac Dougall and Rhodes, 1972). Ledward (1971) reported an antagonism between oxygen presence and MRA, with rapid depletion of MRA in aerobic systems.

Electrical stimulation (ES) can affect MRA. Sleper (1982) found ES carcasses to have a lower anaerobic MRA at 10 d post mortem. This could be due to a different pH behaviour, or to a lowered OCR, which would account for a larger oxygen availability in the tissue. No differences were reported in aerobic MRA at 15 d post mortem.

Stewart et al., (1965b) found a positive correlation between MRA and muscle total pigment content.

pH AND OPTICAL PROPERTIES OF MEAT SURFACES

Immediately after slaughter, muscle is translucent and dark in appearance (Mac Dougall, 1982). This translucence is progressively altered during rigor mortis development, and provided that the muscle has enough glycogen as to allow a large enough pH decline, muscle opacity increases, until it reaches the state in which, after oxygenation, a cut surface has its characteristic bright red color (Mac Dougall and Jones, 1980). The boundary lies at approximately pH 5.9.

If not enough glycogen is available at the moment of slaughter, the normal pH decline is not possible, since the substrate for the glycolytic pathway is lacking. This lessened pH drop will affect the color of meat, both by increasing its oxygen uptake (Lawrie, 1958; Bendall, 1972; Urbin and Wilson, 1961), and raising the MRA (Giddings, 1977; Cutaia and Ordal, 1964; Stewart et al., 1965b).

At a high pH, muscle proteins are far from their isoelectric pH, and their water holding capacity (WHC) is larger. Muscle fibers are swollen, and tightly

packed together, thus impairing oxygen diffusion into the underlying tissue (Lawrie, 1958).

Perhaps the most important characteristics of dark-cutting muscle with regards to its appearance, are its light scattering properties. When the pH approaches the isoelectric point of muscle proteins, muscle is semi-opaque and scatters more light, having a paler appearance (Mac Dougall, 1977). Mac Dougall (1970) reported a relationship between scatter coefficient and pH. In pork meat, he found scatter coefficient more important than the absorption coefficient in affecting luminous reflectivity.

Translucent meat, with a high ultimate pH, has a scatter coefficient (Smm^{-1}) <0.1; while normal meat has a S mm^{-1} of 0.15 to 0.25 (Mac Dougall, 1977).

Mac Dougall and Rhodes (1972) found high final pH to produce less lightness and saturation. The relationship of lightness and brightness (E) to pH, was linear and negative ($E= (L^2 + S^2)^{1/2}$ where L= lightness and S= saturation). Ultimate pH has been considered responsible of the darker color appearance of meat from intact males. In normal bulls, meat color was related to pigment content. In high pH meats, however, color darkness was caused by pH.

Krzywicky (1979) suggests that high ultimate pH meat, with its increased translucence, will allow light to penetrate deeper into the tissue (beyond the oxymyoglobin layer). Thus, the deep pigment layers would absorb more light, giving the meat a darker appearance, and would reflect it with the spectral characteristics of reduced myoglobin (Mac Dougall and Jones, 1980).

EFFECT OF FREEZING ON MEAT COLOR

Kropf (1971) discussed the advantages and disadvantages of marketing frozen retail cuts. He also made an extensive review of frozen meat marketing problems (Kropf, 1982). The following modifications that occur during freezing, are capable of affecting meat color:

1) Modification of the permeability properties of the packaging film;

2) Mechanical trauma to the tissues by crystal formation, which is determined by the freezing rate (Calvelo, 1981; Love, 1958 and Love and Haraldson, 1961);

3) Solute concentration with osmotic alteration of the extra- and intra-cellular spaces;

4) Increased reaction rate between meat components at low temperatures (Fennema, 1973);and

5) Surface dehydration (Freezer burn).

Mac Dougall (1982), listed the main factors affecting the color of frozen meat as being the freezing rate, intensity of light during display, and packaging method.

Freezing rate was found as a very important factor by numerous researchers. Kropf (1982) quotes results from Ramsbottom (1949) showing the effect of freezing rate on color. There is general agreement on the fact that rapid freezing gives the meat a brighter and more natural color. However, at extremely low temperatures, severe bleaching or whitening of the meat has been reported (Sandberg, 1970). This is related to the size of ice crystals, rapid

freezing resulting in very small ice crystals and in more light scattering (Mac Dougall (1974).

Allowing the meat to bloom before freezing improves the appearance of frozen meat (Lentz, 1971). Kropf and Smith (1973) found no differences for beef LD packaged after or before freezing, with different blooming times. PM, however, was brighter when frozen before packaging. Blooming times of less than 30 m, adversely affected the color of PM muscle when packaged before freezing. Pierson et al. (1970) found the reduction time of myoglobin after vacuum packaging to increase as the time period between cutting and packaging was increased. Zaccharіah and Satterlee (1973) measured temperature effect for purified myoglobin, and found highest intensity of discoloration at -12 C, and lowest when below -18 C.

DISPLAY LIGHTING

Solberg and Franke (1971), found illuminated samples to have 5.5% more MMB than samples kept in the dark.

According to Kropf (1980), lighting effect could be exerted by one or more of the following mechanisms: 1) Temperature elevation of the meat surface; 2) photochemical effect , and 3) differences in color rendition. Display temperature was shown to affect frozen meat color stability by Sandberg (1970), Hunt et al. (1975), and Santamaria (1970). The light source used for display, along with the lighting intensity, plays an important role in display life and on the surface temperature of the meat.

Mac Dougall (1982) considered photooxidation of the pigment as the major problem in color stability of frozen meat. Thus, myoglobin oxidation occurs in an opposite direction as in fresh beef, that is, it happens from the surface inwards.

Meat discoloration is stimulated by light. Lentz (1971), found frozen beef color attractive for three months in the dark, but only for three days in the light. Light discoloration has been found by Santamaria (1970), Leising (1976), Schafer (1972) and Fry (1972). Zacchariah and Satterlee (1973), studying the effect of different light sources on Mb autooxidation, found short wavelength lights yielding the largest autoxidation rate constant.

Setser et al. (1973), found a significant effect from radiant energy when comparing control vs. exposed samples. An interaction was found between atmosphere (oxygen level) and discoloration.

According to Solberg and Franke(1971) this photosensitivity is due to a photochemical activation of a riboflavin-like compound, which would then mediate the oxidation of heme pigments.

TABLE 1. SUMMARY OF U. S. STUDIES COMPARING CARCASS COMPOSITION PARAMETERS FROM YOUNG BULLS AND STEERS

Trait	Young Bulls	Steers	Difference
Carcass Wt. (lbs)	621	590	31
12th rib fat	.42	.65	.23
Loin eye area (in^2)	12.57	10.65	1.92
Yield grade	2.0	3.4	1.4
% Separable Lean (Boneless Closely trimmed Retail Cuts)	66.92	51.82	5.1
% Carcass bone	15.4	15.2	.2
Loin eye area/cwt.(in^2)	2.02	1.80	.22
12th rib fat/cwt.(in)	.07	.11	.04
Dressing percent	61.7	62.2	.5

From Allen (1982)

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CHAPTER III

DISPLAY COLOR STABILITY OF FROZEN LONGISSIMUS AND SPINALIS DORSI FROM BULLS AND ZERANOL IMPLANTED BULLS AND STEERS

INTRODUCTION

Beef production from bulls is receiving considerable interest due to their definite advantages in performance and feed efficiency. Some disadvantages, however, prevent the more extensive utilization of bulls for meat production. Among these, darker color of bull meat (Seideman, 1982; Field, 1971) is one of the most important. Ralgro implants alter some characteristics of bull carcasses (Greathouse, 1982; Gray et al., 1983; Unruh et al., 1983), making them resemble steer carcasses in some traits. Some of these traits may influence lean meat color.

Color affects consumer selection, thus influencing the experiencing of other meat characteristics (Jacobs et al., 1977; Kropf, 1980 Frozen retail marketing offers some definite advantages to the meat industry (Kropf, 1982), and its application may increase in the future.

The purpose of this paper is to compare bulls, Zeranol implanted bulls and steers for color characteristics of frozen rib steaks (longissimus and spinalis dorsi muscles) under display conditions. Longissimus and spinalis dorsi muscles

may be a good model for "white" and "red" muscles for display studies of beef, with the added advantage of being located in the same retail cut.

MATERIALS AND METHODS

Fifty five Simmental cross-bred bull calves were randomly assigned to one of five treatments; not implanted (Bulls), implanted at birth and castrated at five months (Steers), bulls implanted from birth to slaughter (IBS), bulls implanted from birth to weaning (IBW), and bulls implanted from weaning to slaughter (IWS). Subcutaneous implanting was done with 36 mg of Zeranol each 100 d. Weaning was at 250 d, and slaughter was at 17 mo for all the treatments, after feeding on a diet consisting of 28.3% corn (Ref No 4-02-931), 56.2% sorghum silage (Ref No 3-04-468) and 5.5% of a mineral - vitamin - chlortetracycline supplement for 259 d.

All carcasses were electrically stimulated (23 impulses of 550 V, 2 s on and one s off), chilled for 24 h and graded. Primal ribs were removed between 24 and 36 h post mortem, and transferred to the Meats Laboratory of the Department of Animal Sciences and Industry, Kansas State University , where they were aged for 7 d at 4 C . One steak from the 8th thoracic vertebral location of approximately 2.5 cm in thickness was removed and vacuum packaged in a Bivac packager, using Iolon film (Oxygen permeability: 210 $cm^3 O_2 \cdot m^2 \cdot 24h \cdot atm$ @ 22.7 C.

After packaging, steaks were placed in a still freezer at -20.6 C for 24 h. Frozen steaks were displayed in an open top case at -17.8 C with twice daily defrost, under GE Natural fluorescent lighting, with a continuous intensity of 968 lux (24 h daily).

Color evaluation was done on day 0 (before display) and at 3, 7, 14 and 21 d for both longissimus (LD) and spinalis dorsi (SD) muscles. Steaks were independently scored by a five member trained panel to the nearest half unit using the KSU visual color scale (1= very bright red, 2= bright red, 3= slight dark red or brown, 4= dark red or brown, and 5= extremely dark red or brown). The panel evaluated both "average" surface color, and "worst" point score which was defined as a single or cumulative discolored area of at least 2 cm in diameter. When no "worst" point was found, the same score was assigned as for average area.

Objective color measurements were done with a Hunterlab D54 reflectance spectrophotometer. In order to estimate proportions of each chemical form of the pigment, reflectance data were converted to K/S values (Hunt, 1980), using the formula: $K/S=(1-R^2)/2R$, where R is the percent reflectance at a given wavelength, expressed as a decimal fraction. K/S calculation brings along an inversion of the data, so that for any pigment form a larger value of K/S means a smaller amount of that pigment form, and viceversa. K/S 630 nm minus K/S 580 nm was used as an estimator of oxymyoglobin content (OMB), the ratio K/S 572 nm / K/S 525 nm as an estimator of metmyoglobin (MMB), and K/S 474 nm / K/S 525 nm as an estimator of reduced myoglobin (MB).

Photochemical effect was determined by the difference in percent reflectance at 630 nm minus the percent reflectance at 580 nm between the exposed (top) and the unexposed (bottom) surfaces of the steaks at 7 and 14 d.

Data were subjected to analysis of variance as a split-split plot design, using "animal within treatment" as the error term for treatment effect, and the interaction "animal by muscle within treatment"as an error term for muscle effect and for treatment by muscle interaction. Least significant differences were calculated according to Cochran and Cox (1957). All data were processed using the Statistical Analysis System (SAS Institute, 1983).

RESULTS AND DISCUSSION

Table 1 shows probability of variation contributed by main effects and their interactions for characteristics evaluated in this study. Table 2 shows the mean visual color scores (mean of LD and SD) for the five treatments. At day 0 (before display), steaks from steers and IWS were brighter than those from bulls and IBS or IBW. On these same animals, Gray et al. (1983) also found brighter lean color at 24 h post-mortem in longissimus from steers than from bulls, lean color for the implant treatments had intermediate values.

Bull steaks remained darker than steer steaks at day 3 of display. By day 7, however, existing differences were masked by massive discoloration. These results are in agreement with the evidence presented in the literature showing

bull meat to be darker than steer meat (Arthaud et al., 1969; Field, 1971; Arthaud et al., 1977; Jacobs et al., 1977; Cross and Allen, 1982; Smith, 1982 and Seideman et al., 1982).

If we consider a 4 score on the KSU scale as an arbitrary limit of acceptability, LD color remained acceptable until day 3 (mean of the five treatments, since no treatment by muscle interaction was present), while SD color was acceptable only at day 0, before display.

The dark color observed since very early display can be explained both by photochemical effect (Solberg and Franke, 1971; Lentz, 1971; Kropf, 1980; Kropf, 1982) and by the relatively slow freezing rate (Sandberg, 1970; Mac Dougall, 1974; Kropf, 1982).

There were important muscle differences, LD being brighter than SD at days 0 and 3 of display. Tuma (1971) found myoglobin content of SD higher than LD. Freezing after packaging may have affected selectively the color of SD , since freezing affected psoas major (a red muscle) more than LD (Sandberg, 1970; Schafer, 1972; Kropf and Smith, 1973; Leising, 1976) . Packaging, even with the moderately O_2 permeable film will put some restriction in the O_2 availability for red muscles which have higher O_2 requirements.

Higher metmyoglobin reducing activity (MRA) has been associated with greater total muscle pigment (Stewart et al., 1965). This fact would explain the dark-purplish color of the SD muscle, as well as its apparent greater stability. Although the scoring system does not show the difference, LD discoloration was more of the opaque brown of metmyoglobin, while SD was always of a dark

purplish-red to black color, suggesting a greater color contribution from reduced pigment.

Pigment estimators for the implanted groups (table 3), show no treatment effect on MB, but bulls and IBW had more metmyoglobin (MMB) than Steers and IBS, IWS being intermediate. For oxymyoglobin (OMB), steers had the higher content, while bulls had the lowest, the implanted groups being again intermediate. IBS group was closer to steers in OMB content than any other implanted group. This supports visual observations by Gray et al. (1983) and Unruh et al. (1983) on the same animals of this study.

SD averaged more MB (lower ratio), less MMB (higher ratio) and more OMB (smaller difference) than longissimius during display (table 4). During display, MB was decreased for SD, while it increased for LD. MMB content increased (lower ratio) up to day 7 and decreased on days 14 and 21 for LD. For SD, MMB showed a constant increase during display. OMB, while it kept decreasing (higher difference) in SD, sharply decreased in LD until day 7, showing then a slight increase during days 14 and 21.

Incidence of double toning, calculated as the difference of the average visual score minus the "worst" point color score, was higher in the beginning of the display period for bulls and IWS (table 5) at the beginning of the display period . Further discoloration, however, reduced the differences. Muscle differences were present in double toning, SD having less at the beginning of display, and being more consistent during display than LD.

Photochemical effect on displayed muscles was compared at days 7 and 14 of display (table 6), by use of reflectance difference of top (light exposed) and bottom (non-light exposed) surfaces of the muscles. It was higher for LD

than SD at 7 d of display. By 14 d of display, difference decreased for LD, meaning discoloration of the unexposed surface was greater for LD than for SD, which maintained the same value as at 7 d. This indicates that photochemical effect is a highly muscle-dependent characteristic.

TABLE 1. PROBABILITY VALUES[a] FOR THE VARIABLES STUDIED

Source of Variation	Visual Score	Ave. Differ.[b]	MB[c]	MMB[d]	OMB[d]
Treatment(T)	.3844	.1660	.2151	.0100	.0489
Muscle (M)	.0001	.0001	.0001	.0001	.0003
Day	.0001	.0001	.0001	.0001	.0001
T * M	.2888	.1060	.0245	.1338	.3489
T .* Day	.0001	.0356	.4518	.0771	.1038
M * Day	.0001	.0001	.0001	.0001	.0001
T *M *Day	.6428	.6833	.6387	.2897	.7085

[a] Probability of a more deviant value, sign ignored.

[b] Avg visual score minus worst point visual score.

[c] Reduced myoglobin estimated by K/S at 474 nm / K/S at 525 nm.

[d] Metmyoglobin estimated by K/S at 572 nm / K/S at 525 nm.

[e] Oxymyoglobin estimated by K/S at 630 nm / K/S at 580 nm.

TABLE 2. MEAN VISUAL COLOR SCORES[a] FOR TREATMENT GROUPS AND MUSCLES DURING DISPLAY

Treatment	Day of display				
	0	3	7	14	21
Bulls	3.32^{by}	4.26^{cy}	4.38^{dx}	4.54^{ex}	4.52^{ex}
IBS	3.34^{by}	4.12^{cxy}	4.42^{dx}	4.59^{ex}	4.48^{dx}
IBW	3.31^{by}	4.03^{cx}	4.47^{dx}	4.57^{ex}	4.54^{dex}
IWS	3.11^{bx}	4.04^{cx}	4.42^{dx}	4.59^{ex}	4.57^{ex}
Steers	3.17^{bx}	4.00^{cx}	4.34^{dx}	4.50^{ex}	4.44^{ex}
Muscle					
LD	2.66^{b}	3.92^{c}	4.44^{d}	4.54^{d}	4.51^{d}
SD	3.84^{b}	4.26^{c}	4.37^{cd}	4.57^{d}	4.51^{d}
Mean	3.25^{b}	4.09^{c}	4.40^{d}	4.55^{e}	4.51^{e}

[a] KSU color scale: 1= Very bright red, 2= bright red, 3= Sl. dark red or brown, 4= dark red or brown, 5= extremely dark red or brown.

b,c,d,e Means in the same row with same superscript letter are not different ($p>.05$).

x,y,z Means in the same column with same supersript letter are not different ($p>.05$).

TABLE 3. MEAN PIGMENT ESTIMATORS FOR THE IMPLANTED GROUPS FOR COMBINED MUSCLES

K/S	BULLS	IBS	IBW	IWS	STEERS
MB[ad]	.94[e]	.93[e]	.93[e]	.94[e]	.95[e]
MMB[bd]	1.00[e]	1.03[fg]	1.00[e]	1.01[ef]	1.03[g]
OMB[cd]	.47[g]	.44[ef]	.46[fg]	.46[efg]	.43[e]

[a] Reduced myoglobin estimated by K/S 474 / K/S 525.

[b] Metmyoglobin estimated by K/S 572 / K/S 525.

[c] Oxymyoglobin estimated by K/S 630 / K/S 580.

[d] K/S values are inverted, i.e., a larger value means less pigment content.

[e,f,g] Means in the same row with same superscript letter are not different (P>.05).

TABLE 4. MEAN PIGMENT ESTIMATORS FOR LONGISSIMUS (LD) AND SPINALIS DORSI (SD) MUSCLES DURING DISPLAY

Pigment Form	Day of display 0	3	7	14	21	Avg
MB,LD[ad]	.95[e]	.95[e]	.96[e]	.93[f]	.93[f]	.95
MB,SD	.93[e]	.92[e]	.93[e]	.92[e]	.94[f]	.93*
MMB,LD[bd]	.98[i]	.96[h]	.88[e]	.90[f]	.94[g]	.97
MMB,SD	1.13[i]	1.08[h]	1.05[g]	1.03[f]	1.01[e]	1.06*
OMB,LD[cd]	.16[e]	.48[f]	.60[i]	.53[g]	.55[h]	.46
OMB,SD	.34[e]	.41[f]	.45[g]	.45[g]	.52[h]	.43*

[a] Myoglobin estimated by K/S 474 / K/S 525.

[b] Metmyoglobin estimated by K/S 572 / K/S 525.

[c] Oxymyoglobin estimated by K/S 630 - K/S 580.

[d] K/S values are inverted, i.e., a larger value means less pigment content.

[f,g,h,i,j] Means in the same row with different superscript are different ($p<.05$).

* = ($p<.05$).

TABLE 5. INCIDENCE OF DOUBLE TONING[a] FOR IMPLANTED GROUPS AND MUSCLES DURING DISPLAY

Treatment (Combined muscles)	Day				
	0	3	7	14	21
Bulls	$.39^{dxy}$	$.17^{bcx}$	$.21^{cx}$	$.24^{cx}$	$.13^{bx}$
IBS	$.31^{cx}$	$.18^{bx}$	$.21^{bx}$	$.16^{bx}$	$.14^{bx}$
IBW	$.33^{dx}$	$.30^{cdy}$	$.23^{cx}$	$.23^{cx}$	$.16^{bx}$
IWS	$.43^{dy}$	$.18^{bcx}$	$.21^{cx}$	$.19^{bcx}$	$.13^{bx}$
Steer	$.34^{dx}$	$.17^{bcx}$	$.19^{cx}$	$.19^{cx}$	$.11^{bx}$
Muscle (Combined treatments)					
LD	$.48^{c}$	$.23^{b}$	$.24^{b}$	$.26^{b}$	$.19^{b}$
SD	$.24^{c}$	$.17^{c}$	$.18^{c}$	$.14^{c}$	$.08^{b}$

[a] Double toning = Visual avg score minus visual "worst point"score.

b,c,d. Means in the same row with the same superscript letter are not different ($p>.05$).

x,y,z Means in the same column with the same superscript letter are not different ($p>.05$).

TABLE 6. PHOTOCHEMICAL EFFECT[a] ON LONGISSIMUS (LD) AND SPINALIS DORSI (SD) MUSCLES

Muscle	Day7	Day14	Avg
LD	-6.74[c]	-4.70[b]	-5.73**
SD	-.49[b]	.04[b]	-.02
BOTH	-3.64**	-2.37	

[a] Top minus bottom surface readings, %R630-%R580

** P <.01
* P <.05
[b,c] means in the same row with different superscript are different (p<.05)

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CHAPTER IV

DISPLAY COLOR STABILITY OF LONGISSIMUS AND SPINALIS DORSI FROM BULLS AND ZERANOL IMPLANTED BULLS AND STEERS WHEN RIB STEAKS ARE PACKAGED IN HIGH OXYGEN-BARRIER FILM

INTRODUCTION

There is considerable interest in bull meat characteristics, since bulls gain more rapidly and are more efficient for beef production (Klostermann et al., 1954; Hedrick et al., 1969), as well as have higher retail yields than steers (Jacobs et al, 1977a; Arthaud et al., 1969; Allen, 1982). Bull meat, however, has darker color than steer meat (Seideman et al, 1982; Field, 1971). Color is one of the most important characteristics affecting consumer preference and meat marketing (Jacobs et al., 1977b; Kropf, 1980). Zeranol implanting of bulls, however, modifies to some extent the characteristics of bull carcasses, affecting some traits that might improve lean meat color (Greathouse, 1982; Greathouse et al., 1983; Gray et al., 1983; Unruh et al., 1983)

In previous studies on these same animals, steers had brighter lean color than bulls, either at 24 h post-mortem (Gray et al.,1983), or when frozen displayed packaged in oxygen permeable film (Chapter III), with the implanted groups having intermediate characteristics.

Vacuum packaging of fresh beef retail cuts in high oxygen-barrier film increased storage and display life which may be very useful with the increase of centrallized packaging and fabrication. Although the consumer may need education to accept the purple red myoglobin color, this seems not to be a critical factor in marketing (Ernst, 1980). This kind of packaging offers longer meat display life (Claus 1981, Griffin et al. 1982).

The purpose of this paper is to compare the color and display life of steaks from bulls, Zeranol implanted bulls and steers when packaged in high oxygen-barrier film.

MATERIALS AND METHODS

Fifty five Simmental cross-bred bull calves were randomly assigned to one of five treatments; not implanted (bulls), Implanted and castrated at five months (steers), bulls implanted from birth to slaughter (IBS), bulls implanted from birth to weaning (IBW), and bulls implanted from weaning to slaughter (IWS). Subcutaneous implanting with 36 mg of Zeranol was done each 100 d. Weaning was at 250 d, and slaughter was at 17 m for all the treatments, after feeding on a diet consisting of 28.3% corn (Ref No 4-02-931), 56.2% sorghum silage (Ref No 3-04-468) and 5.5% of a mineral - vitamin - chlortetracycline supplement for 259 d.

All carcasses were electrically stimulated (23 pulses of 550 V. 2 s on and 1 s off), chilled for 24 h, ribbed and graded. Primal rib cuts were removed at

between 24 and 36 h post mortem , and transferred to the Meats Laboratory of the Department of Animal Science, Kansas State University, where they were aged for 7 d at 4 C. One steak from the 8th thoracic vertebral location approximately 2.5 cm thick was removed and skin-tight vacuum packaged with a Bivac packager using skin-tight Saran-surlyn high oxygen-barrier film , 3.3 mil base, and a 5.2 mil top web (oxygen transmission rate < $10cm^3 \cdot m^2 \cdot 24$ h. @ 23 C, and kept in the dark for 24 h to allow complete myoglobin reduction. Steaks were then displayed at 2 C in open top cases with twice daily defrost, under 968 lux of continuous (24 h/day) GE natural fluorescent lighting.

Color evaluation was done on day 0 (before display) and after 3, 7, 14 and 21 d of display for both longissimus and spinalis dorsi muscles. A five member trained panel independently scored steaks to the nearest 0.5 on the KSU visual color scale (1= bright purple red, 2= dull purple red, 3= slightly brownish purple red, 4= brownish purple red, and 5= brown; Claus, 1982). Panelists were required to score both the average surface color, and the "worst" point color. This was defined as any single or cumulative area of greater discoloration of at least 2 cm in diameter, and was intended to quantify uneven surface discoloration (double toning). If no "worst" point color was observed, average and worst point scores were equal.

Objective color measurements were done with a Hunterlab D54 reflectance spectrophotometer, with the exception of day 14 due to equipment problems. Conversion of reflectance data to K/S values was done using the formula; $K/S=(1-R^2)/2R$, where R is the percent reflectance at a given wavelength, expressed as a decimal . K/S calculation results in an inversion of

the data, so that for any pigment form, a larger value of K/S means a smaller percentage of that pigment form, and vice versa. K/S 630 nm minus K/S 580 nm was used as an estimator of oxymyoglobin content (OMB), the ratio K/S 572 nm / K/S 525 nm as an estimator of metmyoglobin (MMB), and K/S 474 nm / K/S 525 nm as an estimator of reduced myoglobin (MB).

After the 21 d display period, five steaks from each treatment group were randomly selected for blooming studies. Packages were opened, and after an informal evaluation for off odor, spectrophotometric pigment estimations were made, using the same K/S ratios and differences as during display. Pigment estimators were obtained at 0 time (before opening the package) and at 5, 15 and 30 min after opening the package.

The statistical analysis performed was an analysis of variance as a split-split plot design, using animal by treatment as the error term for treatment effect, and the interaction animal by muscle within treatment as an error term for muscle effect and for treatment by muscle interaction. Least significant differences were calculated according to Cochran and Cox (1957). All data were processed using the Statistical Analysis System (SAS Institute, 1983).

RESULTS AND DISCUSSION

Table 1 shows probability for variation contributed by main effects and their interactions for characteristics evaluated in this study. Table 2 shows the mean visual color scores during display of steaks from the five treatment

groups. Longissimus and spinalis dorsi muscles from steers had a duller purplish-red color ($p<.05$) than from bulls and implanted bulls, and this difference continued through day 14 of display. Visual scores remained acceptable (limit of acceptability arbitrarily set at 4 on the KSU scale), during the 21 d of display for the five treatment groups. This acceptability is in agreement with data from Griffin et al. (1982) and Claus (1982) for steaks packaged in high oxygen-barrier film.

The relative color score for the treatments is the inverse of the scores of the same animals when packaged in O_2 permeable film (chapter III), where steaks from bulls were scored darker ($p<.05$) than steaks from steers. When muscles are placed in an anoxic environment as in this experiment, all pigment forms are likely converted to MB at a rate dependant on muscle metmyoglobin reducing activity (MRA), time and effectiveness of oxygen evacuation. Steer muscles may have less MRA, and may also have taken up more oxygen before they were packaged, resulting in the less desirable, duller, purplish red score.

Table 3 shows mean pigment estimator averages. Since K/S calculations inverted the data, a lower value indicates a higher pigment form proportion. Steers and IBS groups had a lower reduced myoglobin (MB) content (higher ratio) than bulls and the other treatment groups, suggesting a lower MRA.

Purser (1982) found higher pH values in bull than in steer meat samples, and Mac Dougall and Rhodes (1972) found the darker color of bull meat to be associated with higher pH values. High pH would increase the oxygen uptake of muscle (Lawrie, 1958; Urbin and Wilson, 1961; Bendall and Taylor, 1972) and its MRA (Cutaia and Ordal, 1964; Stewart et al., 1965), thus narrowing the

oxymyoglobin layer, and bringing the metmyoglobin layer closer to the surface. Impaired O_2 diffusion into the tissue due to swollen and tightly packed fibers of higher pH muscle, would contribute to these effects (Lawrie, 1958). Increased translucence of high pH meat would allow more light to be reflected from the deep MB layer (Krzywicky, 1979), and this light would have more of the optical characteristics of reduced MB. All these factors could contribute to the darker color of of bull meat, supporting the idea that it is pH associated (Field, 1971; Mac Dougall and Rhodes, 1972; Smith, 1982; Cross and Allen, 1982).

There were no pH data available for our study, but a higher pH would enhance MRA, lowering MMB content and yielding a brighter color. When samples were fabricated, steer meat, with a probably lower pH, had less O_2 consumption and more O_2 diffusion (Lawrie, 1958; Bendall & Taylor, 1972), thus blooming faster and to a greater extent. This faster blooming was observed in this same animals, when allowed to bloom for grading (Unruh, 1983). The oxygen uptake during blooming, has some carryover value in raising package pO_2 (Kropf, 1981). Hence, it might be responsible for a less effective MMB reduction.

Surface double toning, measured as the average visual score minus the "worst" point score, is shown in table 4, both for LD snd SD muscles. For LD muscle, IBS and IWS showed less ($p< .05$) double toning than bulls and the IBW group, while for SD muscle there was no difference between groups. The smaller area of SD muscle may partly account for the more homogeneous appearance.

Table 5 and figures 1, 2 and 3 show the estimators of the different longissimus pigment forms during the blooming study. While table 5 shows K/S values for which a lower number means a higher pigment proportion, the inverse of K/S values was used for the figures, in order to give a less confusing image of the actual proportion of each pigment form of the samples. Steers had the most rapid decrease in MB concentration, especially for the first five minutes (figure 1). MB concentration decrease was so rapid that it was almost completed by five min after opening the package, while the other groups tended to continue more slowly until 15, or even 30 min for the IWS group. Estimators of MMB concentration are shown in figure 2. All the implanted groups showed a sharp decrease of MMB in the first five min after unpackaging and initiation of exposure to oxygen. This decreasing period was extended to 15 min for longissimus from the IBW group. While steer group MMB remained constant after the initial drop, in the other treatments this initial decrease was followed by an increase of this pigment form. In bulls, MMB decreased again after 15 min. Probably oxygen consumption by the tissue started once the aerobic conditions were restored, thus lowering the pO_2 in surface layers of the muscle enough to favor a pigment conversion to MMB. This increase in MMB was particularly important for the IWS group. Figure 3 shows OMB estimators, and closely resembles an inverse image of figure 2. From the behavior of OMB in bull samples, increasing again after having decreased slightly, it can be inferred that the decrease in MMB observed in this treatment after 15 minutes was not due to an increase in MRA, but to a further oxygenation of the pigment. It appears as if the O_2 needs for tissue consumption were fulfilled, thus limiting

the O_2 scavenging and allowing more O_2 to be available for pigment oxygenation.

Steer samples were slower in blooming, and had a lower final OMB concentration than the other treatment groups, with the exception of IWS group. The highest OMB accumulation was found in the IBW group. A possible explanation for the slow blooming of steers, might be in the faster and greater bloom steer carcass muscles had when fabricated, which could lessen later blooming ability to some degree. Pierson et al.(1970) demonstrated the effect of longer aerobic holding on decreasing MRA. This might have been responsible for a less effective MB formation after packaging, with a greater MMB accumulation.

On an informal odor evaluation after opening the packages all steaks had a pronounced lactic acid ("yogurt like") odor. This odor disappeared, however, after a few minutes of exposure to air.

Our data suggest vacuum packaging in high oxygen-barrier film as a convenient way to market bull meat in order to overcome its darker color. Since appearance might be less sensitive to other deteriorative changes, careful monitoring of temperature and of storage and display time is definitely required, in order to avoid microbiological problems.

TABLE 1. PROBABILITY VALUES[a] FOR THE VARIABLES STUDIED

Source of Variation	Visual Score	Ave. Differ.[b]	MB[c]	MMB[d]	OMB[d]
Treatment(T)	.0025	.4043	.0073	.5911	.4770
Muscle (M)	.0001	.1645	.2928	.0507	.1362
Day	.0001	.0514	.0001	.0001	.0001
T * M	.3238	.0345	.2928	.3890	.3207
T * Day	.0049	.4825	.2724	.1589	.5332
M * Day	.2398	.0001	.0008	.0001	.0099
T *M *Day	.9997	.5011	.9103	.9357	.2746

[a] Probability of a more deviant value, sign ignored.

[b] Avg visual score minus worst point visual score.

[c] Reduced myoglobin estimated by K/S at 474 nm / K/S at 525 nm.

[d] Metmyoglobin estimated by K/S at 572 nm / K/S at 525 nm.

[e] Oxymyoglobin estimated by K/S at 630 nm / K/S at 580 nm.

TABLE 2. COMBINED MEAN VISUAL SCORES DURING DISPLAY[a] FOR LONGISIMUS AND SPINALIS DORSI FROM BULLS, IMPLANTED BULLS AND STEERS PACKAGED IN HIGH OXYGEN BARRIER FILM

Treatment	Display day					
	0	3	7	14	21	Avg
Bulls	1.49^{bx}	1.56^{by}	1.74^{bx}	2.31^{cxy}	2.37^{cx}	1.89^{l}
IBS	1.50^{bx}	1.54^{by}	1.75^{bx}	2.32^{cxy}	2.41^{cx}	1.91^{l}
IBW	1.37^{bx}	1.44^{bx}	1.71^{bx}	2.25^{cx}	2.40^{cx}	1.83^{l}
IWS	1.42^{bx}	1.44^{bx}	1.69^{bx}	2.25^{cx}	2.40^{cx}	1.85^{l}
Steers	1.72^{by}	1.78^{bz}	2.01^{bcy}	2.44^{cdy}	2.50^{dx}	2.09^{m}
Day Avg.	1.51^{n}	1.55^{o}	1.78^{p}	2.31^{q}	2.41^{r}	

[a] visual scores: 1= bright purple red, 2= dull purple red, 3= sligtly browish purple red, 4= brownish purple red, and 5= brown.

^{b,c,d} or ^{n,o,p,q} Means in same row with same superscript letter are not different ($p>.05$).

^{x,y,z} or ^{l,m} Means in the same column with the same superscript letter are not different ($p>.05$).

TABLE 3. AVERAGE MEAN PIGMENT ESTIMATORS [a] DURING DISPLAY FOR BULLS AND IMPLANTED BULL AND STEER RIB STEAKS PACKAGED IN HIGH-OXYGEN BARRIER FILM

K/S	Bulls	IBS	IBW	IWS	Steers
MB[be]	.56[f]	.57[g]	.55[f]	.55[f]	.57[g]
MMB[ce]	1.30[f]	1.30[f]	1.31[f]	1.32[f]	1.30[f]
OMB[de]	.24[f]	.24[f]	.23[f]	.25[f]	.24[f]

[a] Both longissimus and spinalis muscles, days 0 to 21 of display.

[b] MB estimated by K/S 474 / K/S 525.

[c] MMB estimated by K/S 572 / K/S 525.

[d] OMB estimated by K/S 630 - K/S 580.

[e] K/S values are inverted, i.e., a larger value means less pigment proportion.

[f,g,h] Means in the same row with same superscript letter are not different ($p>.05$).

TABLE 4. AVERAGE DOUBLE TONING [a] DURING DISPLAY OF BULL, IMPLANTED BULL AND STEER LONGISSIMUS (LD) AND SPINALIS DORSI (SD) MUSCLES PACKAGED IN HIGH OXYGEN-BARRIER FILM

Muscle	Bulls	IBS	IBW	IWS	Steers
LD	.09[c]	.03[b]	.10[c]	.04[b]	.08[bc]
SD	.04[b]	.05[b]	.06[b]	.07[b]	.05[b]

[a] Visual average color score minus "worst" point color score.

[b,c] Means in the same row with the same superscript letter are not different ($p>.05$).

TABLE 5. PIGMENT PREDICTORS OF BULL AND IMPLANTED BULL AND STEER LONGISSIMUS DORSI DURING BLOOMING

Pigment	Treatment	Minutes after unpackaging			
		0	5	15	30
	Bulls	.59^{ex}	.77^{fx}	.84^{gyz}	.85^{gx}
	IBS	.59^{exy}	.77^{fx}	.82^{gxy}	.84^{gx}
MB[ad]	IBW	.61^{ey}	.78^{fx}	.82^{gx}	.84^{hx}
	IWS	.60^{exy}	.77^{fx}	.83^{gxy}	.87^{hy}
	Steers	.60^{exy}	.83^{fy}	.86^{gz}	.87^{gy}
	Bulls	1.24^{ex}	1.34^{gyz}	1.30^{fx}	1.32^{fyz}
	IBS	1.24^{ex}	1.38^{gz}	1.32^{fx}	1.32^{fyz}
MMB[bd]	IBW	1.23^{ex}	1.35^{fyz}	1.40^{gy}	1.36^{fz}
	IWS	1.24^{ex}	1.33^{fy}	1.31^{fx}	1.26^{ex}
	Steers	1.24^{ex}	1.28^{ex}	1.28^{ex}	1.28^{exy}
	Bulls	-2.36^{ex}	-3.67^{fgy}	-3.27^{fxy}	-3.87^{gy}
	IBS	-2.34^{ex}	-4.11^{fyz}	-3.41^{gxy}	-3.50^{gxy}
OMB[cd]	IBW	-2.32^{ex}	-4.47^{fz}	-5.18^{gz}	-4.91^{fgz}
	IWS	-2.38^{ex}	-3.74^{fy}	-3.71^{fy}	-2.75^{gw}
	Steers	-2.14^{ex}	-2.54^{efx}	-2.84^{fx}	-3.14^{fwx}

[a] Reduced myoglobin estimated by K/S 474 / K/S 525
[b] Metmyoglobin estimated by K/S 572 / K/S 525
[c] Oxymyoglobin estimated by K/S 630 - K/S 580
[d] K/S calculation inverts the values, i.e., a larger value means less pigment content.
[e,f,g,h] Means in the same row with same superscript letter are not different ($p>.05$).
[w,x,y,z] Means in the same column within the same pigment estimator with the same letter superscript are not different ($p>.05$).

Figure 1. Estimated reduced myoglobin during blooming.
1 / (K/S 474 / K/S 525).

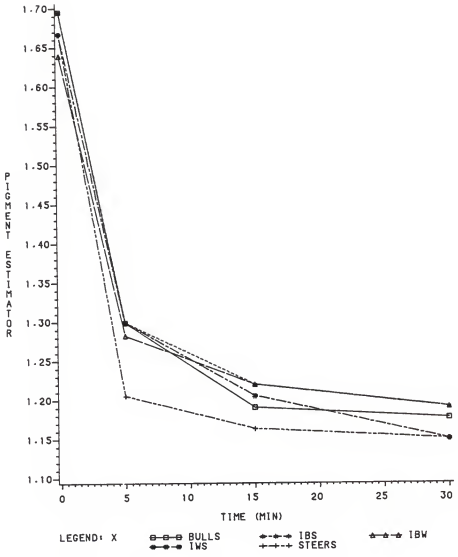

1.70
1.65
1.60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
PIGMENT ESTIMATOR
0
5
10
15
20
25
30
TIME (MIN)
LEGEND: X
BULLS
IWS
IBS
STEERS
IBW

Figure 2. Estimated metmyoglobin during blooming.

1 / (K/S 572 / K/S 525)

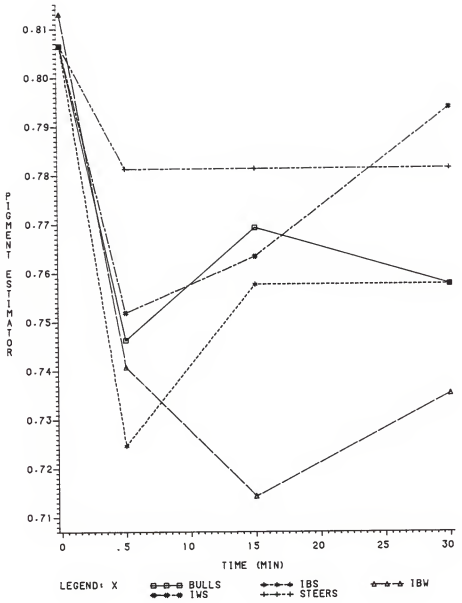
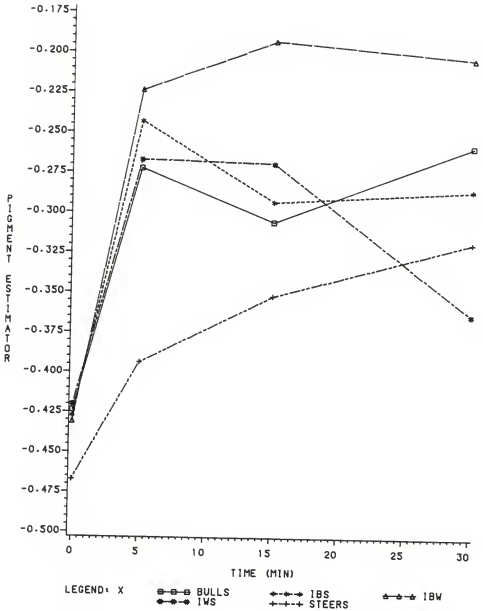

PIGMENT ESTIMATOR
0.81
0.80
0.79
0.78
0.77
0.76
0.75
0.74
0.73
0.72
0.71
0
5
10
15
20
25
30
TIME (MIN)
LEGEND: X
BULLS
IWS
IBS
STEERS
IBW

Figure 3. Estimated oxymyoglobin during blooming.

1 / (K/S 630 - K/S 580)


-0.175
-0.200
-0.225
-0.250
-0.275
-0.300
-0.325
-0.350
-0.375
-0.400
-0.425
-0.450
-0.475
-0.500
PIGMENT ESTIMATOR
0
5
10
15
20
25
30
TIME (MIN)
LEGEND: X
BULLS
IWS
IBS
STEERS
IBW

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CHAPTER V

BEEF COLOR MEASUREMENT: CORRELATION OF SPECTROPHOTOMETRIC REFLECTANCE MEASUREMENTS WITH VISUAL COLOR

INTRODUCTION

Meat color measurement has been extensively reviewed by Hunt (1980). Instrumental methods can be grouped into two basic approaches. Physical description of the perceived color can be obtained by matching that color to a given standard or scale, as in Munsell, CIE-Tristimulus or Hunter-Gardner systems. A second approach to color measurement consists of evaluation of sample pigment properties, thus giving some information about the origin of color deterioration, as with reflectance and transmission spectrophotometry. Detailed descriptions of systems in use are given by Francis and Clydesdale (1975).

Transmission techniques, although adequate for total pigment determinations, are unable to give a true profile of the relative proportion of pigment forms, as they involve extraction procedures which allow an uncontrolled addition of oxygen to the system, thus modifying the relative pigment form estimators (Dean and Ball, 1960).

Hunt (1980) summarized the available information about reflectance at different wavelengths used for measuring meat color. The use of ratios or

differences of reflectance at selected wavelengths, helps to correct for differences in pigment concentrations between samples, as well as for texture or marbling differences. Particularly interesting are measurements of %R474 nm/%R525 nm as an estimator of reduced myoglobin, and of %R572 nm/%R525 nm, as an estimator of metmyoglobin (Snyder, 1964; Stewart et al.,1965a; Ledward, 1970). The ratio of percent reflectance at 630 nm / 525 nm, was used as an estimator of MMb accumulation (Claus, 1982), while %R 630 nm minus %R 580 nm was suggested as an estimator of "redness", or proportion of reduced myoglobin and oxymyoglobin accumulation (Strange et al.,1974; Harrison et al.,1980; Sleper, 1982). Correlations for this ratio with visual score were approximately 0.80.

More recently, Hunt (1982) suggested reflectance at 610 nm as being useful for meat pigment estimations. Reflectance at this particular wavelength is isobestic for Mb and MMB, coincidently with a maximum for OMb. Thus, the %R at 610 nm - %R at 525 nm, as well as %R 610 nm - %R 580 nm and %R610 nm - %R572 nm might be used as estimators of OMb.

Translation of reflectance data into K/S values corrects for some intrinsic optical properties of the sample (Francis and Clydesdale, 1965; Hunt, 1980), linearizing the relationship between reflectance and pigment concentration. Pigment proportion estimates from reflectance data are improved by conversion to K/S values. For the relationship with visual scores, however, Harrison et al.(1980) did not find any improvement in the correlation coefficients to visual color scores when data were converted to K/S values.

Krzywicky (1982) recently proposed using reflex attenuance (log 1/R, Shibata, 1966) as an estimator of muscle pigment concentration, in order to

compensate for the muscle structure optical properties (i.e., the meat absorbtion of light). To account for texture effects, he suggested using the reflex attenuance at 730 nm as a standard, that is, as the muscle completely deprived of pigments. The concept of reflex attenuance is not new, however. Naughton et al. (1958), and Dean and Ball (1960), quoting Wodicka (1956), report using the log of the reciprocal of reflectance (absorbancy) for meat color measurements, although they did not estimate pigment forms.

This study was conducted to evaluate the correlation with visual color scores of some of the most used reflectance measurements and their K/S or log of absorbancy values, and to compare those correlations with the ones obtained when reflectance values at 610 nm are used.

MATERIALS AND METHODS

Two steaks approximately 2.5 cm thick from the 8th thoracic vertebral location of each side of 55 Simmental bull, steer and implanted bull carcasses were fabricated at seven d post-mortem.

One steak from each side was removed, and one of them was vacuum packaged by a Bivac II packager in high oxygen-barrier skin-tight film (Oxygen permeability: $10 cm^3 O_2 \cdot m^2 \cdot 24$ h @ 23 C), and kept in the dark for 24 h to allow complete myoglobin reduction. Steaks were then displayed in an open case at 2 C, under 968 lux of GE natural fluorescent lighting, 24 h daily lighting with twice daily case defrost.

The remaining steak was skin-tight vacuum packaged by a Bivac II packager, using Iolon film (Oxygen permeability: 210 cm^3 $O_2 \cdot m^2 \cdot 24h$ @ 23 C). After packaging, steaks were inmediately placed in a still freezer at -20.6 C for 24 hrs. Frozen steaks were displayed in an open case at -17.8 C with twice daily defrost, under 968 lux of GE Natural fluorescent lighting 24 h daily.

Visual color was independently evaluated by a five member trained panel at days 0 (before display) and at days 3, 7, 14 and 21 of display. The visual color scale used for unfrozen muscles was 1= bright purple red, 2= dull purple red, 3= slightly brownish purple red, 4= brownish purple red, and 5= brown, while for frozen steaks, 1= very bright red, 2= bright red, 3= slightly dark red or brown, 4= dark red or brown, and 5= extremely dark red or brown (Claus, 1982). Spectrophotometric reflectance data were collected on the same display days, with the exeption of day 14 for the unfrozen study, due to equipment problems. Reflectance readings (% reflectance) at 474, 525, 572, 580, 610 and 630 nm were used to calculate the following ratios and/or differences: 630-580, 630/580, 610-525, 610/525, 610-572, 610/572, 474/525, 572/525, 610-580 and 630/525. For all ratios and differences, data were transformed to K/S values using the formula $K/S=(1-R^2)/2R$, where R is the percent reflectance (as a decimal number) at a given wavelength. The same procedure was used with log of absorbancy values, here using the formula Absorbance = 1/R.

Simple linear correlation coefficients were calculated for all the ratios and differences, calculated from %R, K/S and 1/R, using the Statistical Analysis System package (SAS Institute, 1983). In order to compare correlation coefficient magnitudes, Z transformation was done according to Snedecor and

Cochran (1980). For Z values comparisons, the Variance of (Z_1-Z_2), was calculated as follows:

$$\begin{aligned} \text{Var}_{Z_1-Z_2} &= \text{Var } Z_1 + \text{Var } Z_2 - 2 \text{ cov.}(Z_1,Z_2) \\ &< \text{or} = \text{Var } Z_1 + \text{Var } Z_2 \\ &= 1/N-3 + 1/N-3 \\ &= 2/N-3 \end{aligned}$$

Thus, a **conservative** LSD ($p<.05$), which would underestimate rather than overestimate differences was calculated:

Least significant difference (LSD) = $1.96 \sqrt{2 / N-3}$

RESULTS AND DISCUSSION

Simple linear correlation coefficients between visual color scores and ratios or differences of reflectance measurements at different wavelengths, are shown in table I. Although not statistically compared, frozen samples had higher correlation coefficients than unfrozen ones, which might be due to a better reflection from the more opaque frozen surface, or to more definite changes in appearance during display of the frozen steaks (Chapters III and IV). Differences in the predominant pigment forms in both experiments, may also affect correlation coefficients. These data are in agreement with those from Kropf et al. (1970a, 1970b) who found higher correlations for frozen than unfrozen beef. All estimators gave high correlations with frozen reflectance values, with the exception of 474/525. This was reasonable to expect, since reduced myoglobin was not an important pigment form for the frozen muscles in

this experiment. Values for 630/580 are higher than those found by Strange et al. (1974), Leising (1975), Harrison et al. (1980) and Sleper (1982), who reported values of -.86, -.82, -.82 and -.79 respectively. The ratio of reflectance values at 572/525 (a measure of metmyoglobin accumulation) also gave a good although slightly lower correlation of .84, in agreement with the .83 obtained by Harrison et al. (1980). Sleper (1982), however, found a very low correlation for this ratio for polyvinylchloride packaged beef longissimus displayed at 3 C under 1076 lux natural fluorescent lighting and evaluated at 0, 3, 5 and 7 d; but discoloration to high levels of metmyoglobin was minimal in her study.

Estimators that include reflectance at 610 nm (Hunt, 1982), showed a very high correlation with visual scores, comparable to 630 - 580, which would suggest the high potential of using this wavelength.

Transformation of percent reflectance data to K/S did not improve their correlation coefficient with visual scores, agreeing with Leising (1976), Harrison et al. (1980) and Sleper (1982), nor did transformation to "log of absorbancy". Moreover, transformation to K/S values selectively affected the correlation for pigment estimators in which the **difference** of reflectance at two wavelengths was used while it did not affect the correlation with estimators that used the **ratio** of reflectance at 2 wavelengths, i.e., it lowered the correlation coefficient of 630-580, 610-525, 610-572, but did not change correlations with 630/580, 610/525, 610/572, etc.. Correlations using log 1/R were of similar magnitude as those using %R.

In unfrozen samples, correlation coefficients were consistently lower than for frozen. As expected for these samples packaged in O_2 impermeable film, the reduced myoglobin estimator (474/525) shows a much higher correlation

than for frozen samples. Percent reflectance at 630/525, a measurement of metmyoglobin accumulation, had the highest ($p<.05$) correlation value (.61) for unfrozen samples together with 474/525. Because the samples were displayed in an anoxic atmosphere, the low correlation of oxymyoglobin predictors to visual score were expected.

For unfrozen samples, transformation to K/S values increased ($p<.05$) the correlation with visual scores for those estimators obtained by a difference between two wavelengths, while it decreased ($p<.05$) the correlation coefficient when the estimator was obtained using a ratio of K/S values at different wavelength. That is, the transformation increased correlation coefficients at 630-580, 610-525 and 610-572, while decreasing correlation coefficients at 630/580, 610/525 and 610/572. Interestingly, this phenomena is opposed to what was observed for frozen muscles, where K/S transformation decreased correlation of K/S differences, not affecting ratios. A deeper knowledge of the optical properties of meat surfaces and the effect of different pigment forms, as well as of the mathematical manipulations involved, is probably necessary in order to explain this fact.

The use of the logarithm of the absorbance did not result in a significant improvement of correlation coefficients of reflectance data with visual scores, in agreement with Naughton et al. (1957) who found these values interchangeable. The slight apparent increase, however, suggests the need of further research, preferably with the correction for muscle structure suggested by Krzywicky (1979), of using reflectance at 730 nm as the reflectance of the pigment free meat.

TABLE 1. SIMPLE LINEAR CORRELATION COEFFICIENTS OF VISUAL COLOR SCORE WITH REFLECTANCE MEASUREMENTS, K/S AND log 1/R VALUES

Wave-length, nm	Frozen Oxygen permeable %R	K/S	log1/R	Unfrozen Oxygen impermeable %R	K/S	log1/R
630-580	-.92^{ax}	.78^{cy}	.90^{abx}	.33^{dz}	.66^{ax}	.48^{cdey}
630/580	-.91^{ax}	.88^{bx}	-.87^{bcx}	-.55^{abcx}	.09^{dy}	-.61^{abx}
610-525	-.92^{ax}	.57^{dy}	.90^{abx}	.34^{dy}	.61^{ax}	.43^{dey}
610/525	-.90^{ax}	.91^{ax}	-.81^{dx}	-.48^{bcx}	.15^{cdy}	-.56^{abcx}
610-572	-.92^{ax}	.79^{cy}	.91^{ax}	.38^{dy}	.63^{ax}	.47^{cdey}
610/572	-.91^{ax}	.91^{abx}	-.88^{bcy}	-.48^{bcx}	.22^{cdy}	-.58^{abcx}
474/525	.17^{cx}	-.16^{dx}	.17^{ex}	-.57^{abx}	.42^{by}	-.65^{abx}
572/525	.84^{bx}	-.87^{bx}	.84^{cdx}	.42^{cdxy}	-.27^{bcy}	.55^{bcdx}
610-580	-.91^{ax}	.78^{cy}	.90^{abx}	.31^{dy}	.60^{ax}	.34^{ey}
630/525	-.90^{ax}	.91^{ax}	-.84^{cdy}	-.61^{ax}	.41^{by}	-.67^{ax}

^{a,b,c,d,e}: means in the same column with same superscript letter are not different (P>.05)

^{x,y,z} Means in the same row for either frozen or unfrozen samples with the same superscipt letters are not different (P>.05)

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COLOR DISPLAY LIFE UNDER TWO PACKAGING SYSTEMS OF LONGISSIMUS AND SPINALIS DORSI MUSCLES FROM BULLS, ZERANOL IMPLANTED BULLS AND STEERS

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We studied, under two different packaging systems, the display behavior of longissimus and spinalis dorsi muscles from bulls, Zeranol implanted bulls and steers, and calculated the correlation coefficients of various reflectance measurements with visual color scores.

Fifty five Simmental cross-bred bull calves were assigned to one of the following treatments: 1) Not implanted (Bulls); 2) Implanted and castrated at 5 mo (Steers); 3) Implanted from birth to slaughter (IBS); 4) Implanted from birth to weaning (250 days) (IBW) and 5) Implanted from weaning to slaughter. Implantation was done subcutaneously with 36 mg of Zeranol each 100 d, and slaughter for all groups was at 17 mo. The animals were slaughtered, electrically stimulated, and primal rib cuts were aged for 7 d at 4 C. One steak from the 8th vertebral location of each side was removed and a) Vacuum packaged in high-oxygen barrier film (Saran-surlyn), and displayed at 2 C, or b) Vacuum packaged in O_2 permeable film (Iolon), frozen at -20.6 C and displayed at -17.8 C. For both experiments, display was under 968 lux of GE Natural lighting 24 h /d.

Five trained evaluators independently determined "average" and "worst" point color scores at day 0 (before display), and at days 3, 7, 14 and 21 of display. The color scales for the unfrozen was (1= bright purple red, 2= dull purple red, 3= Slightly brownish purple red, 4= brownish purple red, and 5= brown) and for frozen (1= very bright red, 2= bright red, 3= slightly dark red or brown, 4= dark red or brown, and 5= extremely dark red or brown) experiments. Reflectance was measured by a Hunterlab D54 spectrophotometer, K/S 630 - K/S 580 estimating oxymyoglobin (OMB); K/S 572 / K/S 525

estimating metmyoglobin (MMB), and K/S 474 / K/S 525 estimating reduced myoglobin (MB). Susceptibility to light discoloration was measured in the frozen experiment as the difference between the K/S 630 - K/S 580 between the exposed (top) and non-exposed (bottom) surfaces.

In the unfrozen experiment, color of displayed steaks was acceptable until the end of the experiment, steaks from bulls being less discolored than steaks from steers. Zeranol implanting did not affect color scores before or during display, although Steers and IBS had lower estimated MB than the other groups. Double toning, the difference between the "average" and "worst" visual color score, was less for implanted animals than for bulls. When unpackaged, steaks from steers bloomed less than those from other treatments. From their blooming behavior, Bulls and implanted bull muscles appeared to have more oxygen consumption and metmyoglobin reducing activity than steers.

For the frozen experiment, steer steaks were brighter than those from bulls. By day 7, differences were overshadowed by massive discoloration of all treatments. LD muscle remained acceptable until day 3, while SD was only acceptable before display (day 0). Bulls and IBW had more MMB than steers, the other groups being intermediate. OMB was highest in steers and lowest for bulls, IBS being the closest group to Steers. Double toning was higher for Bulls and IWS, until masked by discoloration. LD was more susceptible than SD to photochemical discoloration.

Highest correlations in the frozen experiment between reflectance measurements and visual color appraisal were for % reflectance (at nm) 630-580; 630/580; 610-525; 610/525; 610-572; 610/572; 610-580; and 630/525, all statistically not different and ranging from .90 to .92. Transformation to K/S

or log 1/R values did not improve the correlations. For the unfrozen experiment, highest correlation values were obtained for 630/525 (.61) and 630/580 (.55). When values were transformed to K/S values, highest correlations were for 630-580 (.66); 610-572 (.63) and 610-580 (.60). K/S calculations selectively improved correlation coefficient of reflectance measurements calculated by difference, while it lowered those coefficients when tha calculation was done by a ratio. The inverse phenomena appeared in the frozen experiment, where corelations from ratios were not changed while those from differences were depressed.