MYOGLOBIN PROPERTIES OF ELECTRICALLY STIMULATED BOVINE LONGISSIMUS MUSCLE

by

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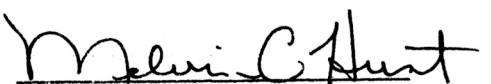

Major Professor

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Chapter I

General Introduction

Small and large meat processors use electrical stimulation (ES) to improve tenderness and lean color, and to facilitate USDA grading and hot boning. At the time of ribbing beef carcasses, improved lean color has been noted for electrically stimulated sides. Little is known, however, about the color phenomenon or the mechanism(s) affecting the color improvement.

Theories relating to color enhancement through electrical stimulation include: 1. ES causes beef to be more pale, soft and exudative, somewhat analogous to PSE pork, 2. ES causes structural damage to muscle that allows deeper oxygen penetration and more light reflection, 3. ES causes increased surface moisture, thus a lighter color is due to increased light reflection, and 4. ES reduces enzymatic activity which competes with myoglobin for oxygen, or alters enzymes affecting myoglobin reduction.

The purpose of this research was to evaluate color of electrically stimulated and not-stimulated meat, visually and instrumentally, and to follow some of muscle's bio-

chemical functions relating to interactions of myoglobin and oxygen.

Chapter III presents the data and conclusions drawn from the electrical stimulation studies. Chapter IV presents a correlation analysis of measurements that are used in meat color study.

Chapter II

Review of Literature

The following abbreviations are used in this chapter:

LD: longissimus dorsi

SM: semimembranosus

ST: semitendinosus

BF: biceps femoris

Mb: reduced myoglobin

MMb: metmyoglobin

MbO: oxymyoglobin

%R: percentage reflectance

R_a: reflectance measured on the absorbance scale

A: absorbance

ES: electrically stimulated

C: control or not-electrically stimulated

MRA: metmyoglobin reducing activity

OCR: oxygen consumption rate

Basic Meat Color

Consumers have color preferences when they purchase beef (Dunsing, 1959; Danner, 1959). "Color is perhaps the most important optical attribute of product appearance" (Clydesdale, 1976). Eagerman et al. (1977) found that even highly trained color panel members had pre-conceived

"ideal meat colors" and panelists rated samples accordingly.

Myoglobin, a sarcoplasmic protein that is water and dilute-salt soluble (Solberg, 1970), is primarily responsible for fresh meat color (Schweigert, 1956). Color intensity differences are obvious when comparing beef, lamb, and pork color. Myoglobin concentration plays a role in color intensity and is a function of: 1. the use of the muscle, 2. the blood supply to the tissue, 3. the oxygen availability, and 4. the animal's age (Clydesdale and Francis, 1971). Hemoglobin, another pigment found in meat, plays a minor role in meat color, but even in some muscles of well bled animals, hemoglobin may be 20-30% of the total meat pigment (Fox, 1966).

Myoglobin has a molecular weight of approximately 17,000 and is considered a heme pigment because it is comprised of a heme moiety attached to the protein globin (Schweigert, 1956). Myoglobin undergoes a series of reactions that create fresh beef color. If the iron in the heme moiety is in the reduced (Fe^{+2}) state and has the sixth ligand associated with water, the pigment will have a purple-red color. When Mb is exposed to air, the sixth ligand position will be oxygenated resulting in MbO (iron is still in the reduced state) which is the desired bright cherry red color of fresh beef. Metmyoglobin is the brown, undesirable form of Mb where the iron is oxidized to the ferric state and the sixth ligand position is

occupied by OH (Schweigert, 1956). Oxygen cannot bind to the heme moiety in the oxidized form (Clydesdale and Francis, 1971). The pigment forms of meat are dependent upon: 1. oxygen tension, 2. temperature, 3. pH, 4. salt concentration, 5. reductant levels, and 6. lighting conditions (Livingston and Brown, 1981).

Unacceptable visual scores for beef have been associated with 50% surface MMb (van den Oord and Wesdorp, 1971b). Greene et al. (1971) reported consumer rejection of meat in the 30 to 40 percent MMb range. Acceptable visual scores for lamb correspond to 25% MMb (Attrey and Sharma, 1979).

Color Measurements

In 1980, Hunt categorized color measurements into the following categories: 1. evaluating a sample's color for selection purposes, 2. grading, 3. consumer responses, 4. marketing specifications, and 5. detecting deteriorative color changes due to processing and storage procedures. There is a need for good color measurement techniques. Color measurement of meat is difficult because several variables influence the results. Absorbance of extracted meat pigments has been used, but this method destroys the sample and does not quantitate the relative amounts of pigment forms due to sample oxygenation or oxidation during the extraction process. Because pigment forms

change with oxygen partial pressures, depth of sampling is another problem of extraction methods. Reflectance measurements of intact meat samples are widely used as a non-destructive method, but problems arise from fat, pH, temperature, area of meat scanned and non-uniformity in color of samples.

Many early color investigators utilized transmission or absorbance of meat extracts to evaluate pigment forms or pigment concentrations. Shenk et al. (1934) proposed using A577/A582 nm of muscle extracts to find relative percentages of hemoglobin and myoglobin. Naughton et al. (1957) used reflectance to study pigments because reflectance analysis was quick and simple, and natural or induced reactions could be followed. Furthermore, heme pigments could be studied when extraction was difficult or impossible due to undesirable pigment changes. The Naughton group used a Beckman DU spectrophotometer equipped with a standard reflectance attachment to study reflectance curves of meat samples.

Pirko and Ayres (1957) used reflectance to study color changes in packaged beef. They reported pigment-free meat had high reflectance at 500-580 nm. In tuna fish meat studies using reflectance spectrophotometry, Naughton et al. (1958) reported that MbO tended to "modify the effect of MMb".

Some of the early important research using absorbancy ratios was conducted by Broumand et al. (1958). They

extracted pigments and proposed using R_a507/R_a573 nm for calculating %MMb; R_a473/R_a597 nm for calculating %Mb; and by difference, calculating %MbO. Their pigment curves were not linear and the pigment forms changed during extraction, thus resulting in errors. Nevertheless, their concept of %R ratios is the basis for many meat color calculations.

Dean and Ball (1960) used reflectance, but applied the Broumand group's ratios to calculate Mb, MMb and MbO on beef samples. When comparing absorbancy ratios to reflectance ratios, they found the absorbancy method gave MbO values that appeared higher than normal.

Snyder (1964) studied ST, SM, and BF muscle samples using a Gardner Color Difference meter. Gardner a values changed appreciably for each pigment form in relation to R_d and b value changes whereas, b values decreased for Mb and MMb, thus differences between MMb and Mb could be determined by using an a/b ratio.

Snyder (1965), using a Beckman DK 2A spectrophotometer, scanned beef samples converted to 100% Mb, MbO or MMb from 400-700 nm using the absorbancy scale to measure reflectance. The resulting curves gave the same maxima as those found by transmission measurements, but the R_a curves were not highly reproducible. Therefore, Snyder adjusted each pigment's reflectance curve by the factor needed to make the curve pass through 1.0 at 525 nm. The adjusted curves gave isobestic points of 474

nm for MMb and MbO, 571 nm for Mb and MbO, and 525 nm for Mb, MMb and MbO. From the adjusted curves, he reported good reproducibility and that ratios of reflectance values were not needed. The adjustment was defended as valid because the curves gave information on percentage values rather than absolute amounts of the pigment forms.

Stewart et al. (1965b) suggested converting reflectance values to K/S values by using Table D of Judd and Wyszecki (1963). A K/S value is the ratio of absorption coefficient to scattering coefficient (Judd and Wyszecki, 1963). Stewart et al. (1965b) reported a linear relationship between K/S values of %R525 nm and total pigment concentration, whereas a curvilinear relationship was plotted using R_a data. Also, a straight line was obtained when plotting the K/S value ratio for 572/525 nm against % MMb. Their K/S constants and others are given in Table 1.

Ledward (1970) reported better consistency in K/S values at 572 nm when R_a of 525 nm was adjusted to 1.0. From the K/S ratio of 572/525 nm, Ledward reported MMb could be calculated within 6 or 7% and Mb within 5%.

Snyder and Armstrong (1967) determined K/S value ratios provided straight lines compared with R_a values which had a curvilinear relationship with data obtained from a model system. Their model system was comprised of nonfat dry milk in which crystallized beef Mb had been suspended. They also compared K/S value ratios from

Table 1-Wavelength and ratio constants reported in the literature

Wavelength or ratio	Pigment form	Constant	Reference
572	Mb, MbO	.56	Stewart et al. (1956b)
525	Mb, MbO, MMb	1.40	Stewart et al. (1956b)
571/525	MbO	1.327	Snyder and Armstrong (1967)
571/525	Mb	1.328	"
571/525	MMb	.608	"
474/525	MbO	.957	"
474/525	Mb	.543	"
474/525	MMb	.962	"
571/525	MMb	.59	Zimmerman and Snyder (1969)
571/525	MbO	1.36	"
474/525	MbO	.88	"
474/525	Mb	.53	"
572/525	MMb	1.450	Ledward (1970)
572/525	Mb	.615	"
474	100%Mb	2.000	"
474	0%Mb	3.880	"
580/525[a]	MbO	1.99	van den Oord and Wesdorp (1971a)
580/525[a]	Mb	.38	"

[a] %R, all other wavelengths or ratios are K/S conversions.

unadjusted spectral data with a single K/S value from pigment curves that had been adjusted to 1.0 at 525 nm. They found no difference in values obtained and suggested using K/S value ratios rather than adjusting reflectance data.

Zimmerman and Snyder (1969) calculated pigment percentages using K/S constants (Table I). They used a Bethlehem No. 614 hyperbaric chamber to subject samples to 80 psig of oxygen for a total of 12 hours per day for 12 days. Samples were completely oxygenated under the high pressure which resulted in MbO K/S ratio constants different from those constants from samples oxygenated in air. No explanation for this phenomena was presented.

The use of an integrating sphere was used to measure total reflectance (Elliott, 1967). Muscle fat elevated percentage reflectance of pork LD muscle; therefore, cross-sectional samples were used to maximize resolution of the spectra because fat is deposited longitudinally along muscle fibers. Plastic films added to light scatter and absorbed light at wavelengths less than 450 nm.

Ockerman and Cahill (1969) attempted to develop a prediction equation that used reflectance values to predict visual color scores. Percentage reflectance at 685 nm provided the best predictor equation for visual scores; however, the equation had an R^2 of .7715 and was inconsistent due mainly to "color panel shift".

van den Oord and Wesdorp (1971a) used a Unicam Sp

800B spectrophotometer equipped with a Unicam SP890 diffuse reflectance accesory to study beef pigments of intact samples. The proportion of light absorbed to that scattered decreases with increasing reflectivity, thus reflectance may not be treated as absorption. They reported that increasing muscle fat content increasingly elevated the whole spectral reflectance curve. Furthermore, they compared %MbO obtained from reflectance and absorbance (pigment extracted) methods and found MbO determined by reflectance was always less than that found by absorbance, which supports the argument that pigment forms change during extraction.

van den Oord and Wesdorp (1971a and 1971b) found absorbance at 580-630 nm to be a good method for following relative changes in proportions of MMb and MbO. Differences at A580-A630 nm were correlated (.94) with visual color panel scores.

Strange et al. (1974) attempted to determine a reliable measurement to replace a color panel. They read samples on a Beckman DU Model 2400 spectrophotometer equipped with an integrating sphere at wavelengths 520, 540, 560, 580, 600, 620, 630, 640, and 700 nm. Gardner a values had a .91 correlation to color panel scores for beef steaks displayed for 5 to 8 days and scored on a hedonic scale. Their three most effective methods for replacing color panels were: 1. the Gardner a value, 2. %R630-%R580, and 3. ln(%R580/%R630).

Physical measurements can "provide a basis for psychophysical scales from which we can predict visual scores" (Little, 1976). Jeremiah et al. (1972) found that Gardner Color Difference meter Rd, a, and b values, Photo-volt 610 reflectance meter readings for blue, green, and amber, and MacBeth-Munsell Disk Colorimeter red, yellow, white, and black disks were significantly related to visual color scores. Hue and value calculated from Munsell standard disks were significantly related to total pigment concentration.

Eagerman et al. (1977) used a highly trained color panel to evaluate beef samples for lightness, hue, chroma, and overall color in attempting to correlate panel scores to instrumental values. When using a Gardner Color Difference meter, however, the best regression equations for pork, beef, and lamb had 20, 40 and 40 % error rates, respectively.

Eagerman et al. (1978) reported that bloom time was always correctly ranked by %R632-%R614 nm obtained from a Bausch and Lomb spectrophotometer with reflectance attachment, but color panels did not consistently correctly rank the samples. Apparently panelists' judgements were affected by more than just color. They proposed using %R632-%R614 nm for a quick method of evaluating samples because samples that were brown and unacceptable had slightly negative differences.

Harrison et al. (1980) correlated various reflectance

measurements to visual color panel scores (Table 2). The %R630-%R580 nm measurement was suggested as a predictor of visual color scores.

Franke and Solberg (1979), working with known amounts of pigment concentrations and reflectance spectrophotometry, read samples for R_a from wavelengths 400 to 750 nm. MMb was measured with a "corrected" R_{a632} nm reading which was calculated by %R632-%R750 nm (750 nm represented a muscle structure reference point). The relationship between ppm total hematin and R_a632 nm was linear. They proposed using 632 nm rather than 425 nm for MMb and MbO determinations because severe non-linearity existed for 0 to 16% MMb at 425 nm. Using R_a632 nm reduced scatter to nonsignificant levels.

Krzywicki (1979) stated that certain assumptions made for using K/S reflectance measurements are not justified. Light penetrates the meat surface, thus the absorbance coefficient is higher than in uniformly pigmented samples. Reflex attenuance (log 1/R) was used to study samples with 730 nm being used as a correction factor for absorption by the pigment-free meat.

Effects of Electrical Stimulation on Meat Color

Benjamin Franklin was probably first to apply electrical stimulation to meat (Lopez and Herbert, 1975). Electrical stimulation was not used commercially until the

Table 2-Objective measurements contributing most to R^2 of equations predicting visual color score of four beef muscles (Harrison et al., 1980)

	Objective measurement	R^2 contribution
Longissimus	%R630 - %R580 nm	0.334
	K/S630 - K/S580 nm	0.018
	K/S630 / K/S580 nm	0.009
	K/S474 / K/S597 nm	0.003
	K/S632 / K/S614 nm	0.008
	%R632 - %R614 nm	0.017
	K/S507 / K/S572 nm	0.003
Semitendinosus	%R582 / %R525 nm	0.179
	%R632 - %R614 nm	0.010
	K/S632 - K/S614 nm	0.041
	%R630 / %R580 nm	0.003
	%R630 - %R580 nm	0.003
	%R507 / %R572 nm	0.003
	K/S474 / K/S597 nm	0.003
	%R572 / %R525 nm	0.002
	K/S507 / K/S572 nm	0.003
Biceps femoris	%R630 - %R580 nm	0.180
	%R630 / %R580 nm	0.002
	K/S630 / K/S580 nm	0.006
	%R582 / %R525 nm	0.002
	K/S632 - K/S614 nm	0.001
	K/S474 / K/S597 nm	0.001
Semimembranosus	%R630 - %R580 nm	0.217
	K/S632 - K/S614 nm	0.011
	%R632 - %R614 nm	0.005
	%R507 / %R572 nm	0.004
	K/S582 / K/S525 nm	0.003

New Zealand lamb industry used electrical stimulation to speed the onset of rigor mortis, thus eliminating cold-induced toughening in muscles of rapidly chilled lamb carcasses. Harsham and Deatherage, (1951) patented a method of electrical stimulation for the purpose of meat tenderization. More recently, researchers have noted that electrical stimulation improved muscle color scores.

Several researchers (Savell et al. 1978b; Savell et al., 1978c; Cross et al., 1979; Calkins et al., 1980; Davis et al., 1981; Salm et al., 1981) have reported improved muscle color at the time of ribbing and after blooming for electrically stimulated LD muscle. Savell et al. (1978b) ribbed carcasses at 19 to 24 hr postmortem and observed electrical stimulation significantly improved muscle color and reduced the incidence of heat ring. When varying impulses per side from 25 to 75, Savell et al. (1978c) found the LD muscle from ES sides of light-weight heifer carcasses ribbed at 24 hr postmortem to be brighter colored than those from C carcasses.

Cross et al. (1979) studied combinations of shrouding and/or PVC film wrapping of ES carcasses. Carcasses were ribbed 18 hr postmortem. Heat ring was less common in ES sides. Shrouded ES carcasses had better muscle color scores than shrouded C carcasses. However, when comparing ES carcasses that were both shrouded and wrapped in PVC film to C carcasses shrouded and PVC film wrapped, no muscle color score differences were found. Control shrouded

and PVC film wrapped carcasses had better muscle color scores than control shrouded sides. They concluded PVC film added little to the effects of electrical stimulation. Seemingly, the slower chilling rate of the shrouded and PVC wrapped carcasses resulted in muscle color similar to the ES beef muscle color.

Savell et al. (1979) evaluated LD muscles from three ES beef sides at a 24 hr postmortem ribbing time and found muscle color uniformity of ES sides was better than for C sides. Calkins et al. (1980) evaluated color at ribbing times of 24, 48 or 72 hr. Overall ES muscle means were brighter and had a lower incidence of heat ring; however, no differences were found in muscle color scores for ES and C sides at 48 or 72 hr. Davis et al. (1981) stimulated carcasses from forage- and grain-finished steers. For all feeding regimens, ES carcasses had brighter muscle color than C carcasses at an 18 to 21 hr postmortem ribbing time.

Salm et al. (1981) compared the effects of electrical stimulation on carcasses from cattle fed high energy diets for different lengths of time. Carcasses ribbed at 24 hr were evaluated for LD muscle color and color uniformity. Electrical stimulation improved muscle color for all diet groups. Without electrical stimulation, animals on low energy diets had inferior muscle color to animals on high energy diets. Muscle color uniformity scores were less variable in ES sides.

McKeith et al. (1980b) varied voltage (150 or 550v), time of stimulation and length of stimulation (1 or 2 min). Cow carcasses were ES before evisceration or after splitting. Electrical stimulation improved muscle color irregardless of stimulation time or voltage, but 550v of stimulation improved muscle color scores and reduced the incidence of heat ring when compared with 150v. Muscle color scores were not different for stimulation times of 1 or 2 min. McKeith et al. (1981) reported similar results when they compared voltages (150 and 550v), time of stimulation and length of stimulation. At the 24 hr postmortem ribbing time, ES LD muscle was always superior to C in muscle color scores and had a lower incidence of heat ring. McKeith et al. (1980a) ES steer and cow carcasses with 550v for 16 impulses of 1.8 sec duration with 1.8 sec intermittent pause between impulses. At ribbing, ES steer LD muscles had brighter color than C steer muscles.

Eikelenboom et al. (1981) stimulated bull carcasses with low voltage (85v of continuous 15 Hz current for 1 min) or with high voltage (300v, 50 Hz of pulsed current; 2.5 sec duration with 1.5 sec interval for 1.5 min). At 24 hr postmortem, an 800 g sample was taken from the 8th to 10th rib region and vacuum packaged. After 7 days storage at 20^{o}C, muscle color and drip loss were evaluated. No differences in Hunterlab L, a or b values were found between low and high voltage ES samples, but ES

samples were significantly lighter in color than C samples. Drip loss was greater for ES than C samples.

Hall et al. (1980) made ground beef (30% fat) from ES and C beef SM and adducter muscles. A 2.5 cm adducter steak and the ground beef were displayed at 1 to 3°C under 1030 lux of incandescent lighting for 4 and 3 days, respectively. A 10 member color panel observed no differences in muscle color, surface discoloration or overall appearance of ES and C ground beef; however, ES adducter steaks were found to be brighter in color than C steaks at 2, 3 and 4 days of display. Surface discoloration for steaks from ES carcasses was less than that for steaks from C caracasses.

Contreras and Harrison (1981) made ground beef from flanks of ES, ES hot boned (ESHB) and C beef. Fat levels were adjusted to 10, 15, and 20%. Samples were stored at -30°C and evaluated for color stability after 2 to 4.5 months of storage. Samples were thawed for 4 hr at 4°C, allowed to bloom 1 hr and then exposed to radiant energy for 4 hr with spectral readings being taken every 30 min. ESHB samples were more sensitive to color changes (%R630-%R580 nm, Hunterlab a, a/b ratios and visual color scores) than ES or C samples.

Cross and Tennent (1980) ES Choice and Good grade beef carcasses and evaluated LD muscle color after 20 days of vacuum storage. Lean maturity for ES Choice grade carcasses was better than for C carcasses, but no other color

differences were found. No differences in water-holding capacity were found between samples from ES and C carcasses.

Orcutt et al. (1981) observed that LD muscle color from ES carcasses was "lighter", and had higher a and b values than control muscle as determined by a Gardner Color Difference meter at 6 and 48 hr postmortem. Spectrophotometrically, the proportion of MbO was not different for ES and control sides. These researchers suggested the lighter appearance of ES meat may be due to deeper oxygen penetration or the ability of the meat surface to reflect more light.

Grusby et al. (1976) ES Standard and Good grade beef carcasses with 320v of 5 amp current for 10 or 20 sec and found no muscle color differences between ES and C sides. Smith et al. (1979) ES unsplit calf carcasses, half with the hide-on and half with the hide-off, with 100v, 5 amps, 50-60 cycles/sec for 50 impulses at 45 to 70 min post-exsanguination. Carcasses were graded at 72 hr and when comparing ES with C hide-on and hide-off control carcasses, no color differences were observed for the LD muscle.

Nichols and Cross (1980) combined hot boning with electrical stimulation and reported that panelists found no differences in muscle color or color uniformity between ES and C LD and SM steaks. Stimulation was at 1 hr postmortem using 1 amp of continuous current in the range of

140 to 200v for 2 min. Muscles were excised immediately after electrical stimulation, 6 hr postmortem or at 5 days postmortem. Steaks were displayed under 88 to 92 ft candles of incandescent light at 3°C for 14 hr/day for 5 days.

Taylor et al. (1981) ES Hereford, Friesian or Hereford X Friesian steer carcasses with 700v of 25 impulses/sec for four 30 sec periods. No differences between ES and C LD and SM muscle color were found for color panel scores and Hunterlab lightness, hue and saturation at 5 or 21 days postmortem.

Retail acceptability of LD steaks from ES bull, heifer and steer carcasses was evaluated by Jeremiah and Martin (1980). Carcasses were stimulated with 400v of 5 amp pulsed (3 to 10 sec duration) current until no additional muscle response was observed. At six days postmortem, LD steaks were displayed under 820 lux of incandescent lighting for 4 days. No color or discoloration differences were found between ES and C steaks within any of the sex groups.

After 6 days of vacuum aging, Kastner et al. (1980) displayed ESHB and C LD steaks under 1076 lux of continuous Delux Warm White fluorescent lighting. No color differences were observed between C and ESHB steaks at 1 or 4 days of display even though ESHB tended to be brighter colored. Stimulation parameters were 400 to 600v and 5 amps of 60 Hz current.

Claus (1982) displayed C, ES, ESHB and hot boned LD steaks for muscle color evaluation at 0, 1, 3, and 5 days. Hot boned steaks were darker colored, but ES and ESHB steaks were similar in color. No differences in MMb were observed between C and ES steaks at 1, 3, or 5 days. At 0 days, ES steaks had less MMb.

Savell et al. (1978a) studied structural changes of ES beef LD. Mean sarcomere lengths did not differ between C and ES samples, but ES samples had less well defined Z-lines and I-bands, and more sarcomere disruption. George et al. (1980) noted that protein denaturation was significantly higher in ES samples than C samples and denaturation was like that found in the PSE condition. Sarcoplasmic proteins were denatured and deposited on myofibrillar proteins; however, water holding capacity was not affected. They concluded that muscle cell membranes of beef were considerably less fragile to conditions of low post-rigor pH than membranes of pig muscle.

Will et al. (1980) observed accelerated autolysis of ES beef LD muscle. At 6 hr postmortem, intercellular edema and flocculant material in connective tissue was observed in ES samples. Whiting et al. (1981) reported that functional (protein solubility) properties of protein from ES lamb muscle were similar to those from C muscle.

Greaser et al. (1969) observed a granular appearance of myofilaments from PSE pork at 24 hr postmortem. Dutson et al. (1974) reported that PSE muscle ultrastructure 15

min postmortem appeared as normal muscle did at 24 hr postmortem.

Forrest and Briskey (1967) reported that LD samples from ES sides of light and heavy weight hogs had lower color scores (.5 = extremely PSE and 5 = extremely DFD) than C sides. Westervelt and Stouffer (1978) also reported lower visual color scores for ES pork LD muscles.

Riley et al. (1980) split wether lamb carcasses and ES the left side for 1 min within 30 min postmortem; the right side was a control. They used 550v, 5 amps, 7 impulses/min with a 1.8 sec duration and a 1.8 sec interval. At 5 days postmortem, boneless LD chops were displayed under 883 lux of incandescent lighting for 4 days. At 24 hr postmortem, muscle color of ES carcasses was better, but a 9 member color panel found no differences for muscle color during display.

Dutson et al. (1980) observed that samples from ES ovine carcasses had more released lysosomal enzymes than control samples, but more of the enzymes in ES samples were degraded due to the combination of higher temperature and pH of ES meat. The left sides of lamb carcasses were stimulated 25 times with 400v, 5 amp pulsed current.

Total Pigment Concentration

Total pigment concentration has a major effect on meat color. Jacobson and Fenton (1956) reported that as

hue increased, so did iron content of LD, PM, and SM muscles. Pigment concentrations reported for beef are given in Table 3. Fleming et al. (1960) observed that total pigment concentration varied depending upon the method of extraction and calculation. Carbon monoxide-pigment complexes provided the most favorable results (Poel, 1949).

The method of Hornsey (1956) is widely used for total pigment quantification and is based on an acidified acetone extraction of total pigments. The method can be used for cured or fresh pigments. Warriss (1979) compared extraction procedures and reported Hornsey's acidified 80% acetone, and the 0.04 M phosphate buffer at pH 6.8 methods extracted the most pigments. Low pH buffer and water methods did not extract as much pigment.

Tang and Henrickson (1980) reported that ES had no effect on total pigment concentration when compared with C beef samples. There was a significant difference between animals and muscles with the SM having the greatest concentration followed by the LD and ST. Taylor et al. (1981) also found no differences in total pigment concentrations of ES and C beef samples.

Oxygenation and Oxidation of Myoglobin

Light, chemicals, gaseous atmosphere, temperature, and pH affect meat color (Solberg, 1968). Temperature,

Table 3-Beef pigment concentrations reported in the literature

Muscle	Pigment	mg/g wet wt	Reference
LD	Mb	2.72	Romans et al. (1965)
LD	Hb	.23	"
LD	Mb	3.18	Rickansrud and Henrickson (1967)
PM	Mb	2.40	"
BF	Mb	3.64	"
ST	Mb	1.99	"
LD	Total	3.97	"
PM	Total	3.84	"
BF	Total	4.85	"
ST	Total	2.89	"
ST	Total	4.6-6.9	Ledward (1970)
LD	Total	3.54	Hunt and Hedrick (1977)
PM	Total	4.07	Hunt and Hedrick (1977)
LD	Total	2.70	Tang and Henrickson (1980)
SM	Total	2.93	"
ST	Total	2.26	"
LD	Total	3.65[a]	Fleming et al. (1960)
LD	Total	3.47[b]	"

[a] As calculated by method of Poel (1949)

[b] As calculated by method of Shenk et al. (1934)

relative humidity, pH, myoglobin concentration, biological agents, gaseous atmosphere, and oxygen penetration are of particular importance to oxygenation of myoglobin and retention of the oxygenated state (Haas and Bratzler, 1965). Solberg (1970) observed oxygenation rate was chiefly affected by temperature and oxygen partial pressure, but as Ashmore (1972) reported, pH indirectly affected oxygenation due to more respiratory enzyme activity at a higher pH. Dark cutting beef consumes more oxygen due to cytochrome enzyme activity thus resulting in decreased oxygen penetration into the meat (Lawrie, 1952). Therefore, less oxygen is available for Mb oxygenation due to enzyme competition. Components of the electron transport chain remain potentially active in meat even after extended refrigeration (Bodwell et al., 1965).

Oxygen penetration depth varies from sample to sample and is important in spectral changes (Pirko and Ayres, 1957). Brooks (1938) reported oxygen penetration of approximately 2 mm in beef while Krzywicki (1979) reported oxygen penetration depth of 3 mm and that pale or bright samples displayed faster oxygenation. Brooks (1929) observed oxygen penetration of 2 mm after a few hours of exposure to air, but 4 mm penetration after 100 hours of exposure to air. He attributed the difference to a slow decrease in oxygen consumption by the tissue. Krzywicki (1979) stated maximum oxygenation was reached in less than 20 min after cutting samples at room temperature. Cutaia

and Ordal (1964) allowed only a 5 min bloom time for ground beef before measuring MbO by reflectance. Their preliminary work indicated a 5 min bloom produced reflectance curves similar to 30 min bloom reflectance curves. Haas and Bratzler (1965), using Munsell Spinning Disks and a Gardner Color Difference meter, found oxygenation was most extensive during the first hour of exposure. As temperature increased, oxygen penetration decreased (Urbin and Wilson, 1961).

Brooks (1931) illustrated the importance of gaseous atmosphere on heme pigments by oxidizing hemoglobin with oxygen. George and Stratmann (1952a) reported some oxygen is needed for MMb formation and the amount of oxidation in a given time is Mb concentration dependent. Using the Warburg apparatus, they found 1.5 moles of oxygen were consumed during the oxidation of 1 mole Mb.

George and Stratmann (1952b) showed that myoglobin oxidation is dependent upon oxygen partial pressure. Maximum oxidation occurred at 1.0 to 1.4. The ability of oxygen to oxidize Mb decreased up to 30 mm oxygen and then became constant. They noted Mb was more susceptible to denaturation than MbO at 38^{o}C and 4 mm of oxygen pressure. MbO is stable as long as the heme is oxygenated, but oxygen continually associates and dissociates from the heme moiety, especially at low oxygen pressures (Clydesdale and Francis, 1971). Ledward (1970) reported maximal MMb formation in beef ST muscle at an oxygen

pressure of 7.5 mm Hg at 7°C and 6 mm at 0°C.

Rikert et al. (1957) reported the rate of discoloration varies inversely with the partial pressure of oxygen. A good vacuum seemed necessary for samples to return to redness (return to Mb) following initial discoloration after packaging. Naughton et al. (1958) noted tuna Mb discolored at low oxygen partial pressures. Meat discoloration is retarded by high oxygen partial pressures (Snyder, 1964; Zimmerman and Snyder, 1969).

Muscle pH has a definite effect upon retention and oxidation of MbO. The lower the pH, the greater the MbO oxidation rate (Brooks, 1931; George and Stratmann, 1954; Fox, 1968; Brown and Mebine, 1969; Solberg, 1970). George and Stratmann, (1954) found the rate of Mb oxidation increased at both low and high oxygen partial pressures as pH fell from 6.44 to 5.35 at 30°C.

Temperature also affects the MbO oxidation rate. As temperature increases, the oxidation rate of MbO increases (Brooks, 1938; Urbin and Wilson, 1958; Snyder, 1964; Brown and Mebine, 1969; van den Oord and Wesdorp, 1971b). MbO oxidation rate may decrease 40 to 50 fold when the temperature is lowered from 22 to -2°C (Brown and Mebine, 1969). Urbin and Wilson (1958) reported a three-fold increase in MMb formation as temperature increased 10°C.

Other factors that contribute to Mb oxidation are bacterial growth and by-products of lipid oxidation. Butler et al. (1953) and Robach and Costilow (1961)

reported bacterial growth increased MMb formation, especially during the logarithmic growth phase, because the microorganisms out competed Mb for existing oxygen and lowered oxygen partial pressures to a level favoring myoglobin oxidation. Greene (1971) found that peroxides, break-down products of lipid oxidation, catalyze pigment oxidation.

Reduction of Ferric Pigments

Brown et al. (1958) concluded the off-color of tuna was due to the heme pigments being in the ferric state. Mb oxidation is a first order reaction (Brown and Dolev, 1963); however, muscle does have a MMb reducing system if the proper conditions are present. Brown and Dolev (1963) noted that crude beef extracts were less susceptible to oxidation than purified Mb.

Two basic approaches have been used for studying MRA. Meat pigments may be oxidized either chemically with $K_3Fe(CN)_6$ or by natural pigment oxidation in low oxygen partial pressures. Following oxidation, the reduction of MMb in either aerobic or anaerobic conditions may be followed spectrophotometrically. Ledward (1972) reported a correlation of .22 between %MMb reduced after chemical oxidation and MMb accumulated on samples after 7 days storage in air. In contrast, he found a -.94 correlation coefficient between MMb accumulated at 7 days

storage in air and %MMb reduced from MMb produced by oxidation in 1% oxygen. However, Ledward allowed a 24 hr reduction time for the 1% oxygen oxidized samples, but only a 1 hr reduction time for the chemically oxidized samples. Ledward stated that chemical oxidation should not be used. Antonini et al. (1965) reported no apparent side effects of pigment oxidation with ferricyanide. Most research in the area of MMb reducing activity has involved a first step of pigment oxidation.

Eagerman et al. (1978) suggested that MMb is always present on the surface of beef. Due to the oxygen partial pressure effect, MMb accumulation after anaerobic packaging has been reported to be 30% in 30 min (Zimmerman and Snyder, 1969) and 40 to 60% in 10 to 15 hr (Cutaia and Ordal, 1964); however, after formation, reduction of MMb was observed. Greene, (1969) noted that anaerobic packaging could be only successful if enough MRA was still present. MRA decreases with decreasing pH (Cutaia and Ordal, 1964; Stewart et al., 1965a) and increases with increasing temperature (Rikert et al., 1957; Cutaia and Ordal, 1964; Stewart et al., 1965a; Hutchins et al., 1967; Solberg, 1968). Stewart et al. (1965a) reported little MRA occurred below 15°C, but rate of MRA increased up to 35°C. They also found a .83 correlation between total pigment concentration and MRA. Ledward (1971) reported rapid MRA depletion by oxygen. In samples thin enough (2mm) for complete oxygen penetration, MMb was not

reduced.

Several researchers have investigated muscle's inherent ability to reduce MMb. Stewart et al. (1965a) proposed a mechanism for MMb reduction involving electron transfer from lactate to NAD by lactic dehydrogenase. The NADH can in turn then reduce MMb. Watts et al. (1966) found addition of NAD could restore MRA and oxygen must be limited for MRA. They noted that electrons from NADH react preferentially with oxygen as long as oxygen remains, thus MMb is not reduced.

Saleh and Watts (1968) reported the possibility that intermediates may transfer electrons from NADH to MMb. Brown and Snyder (1969) found NADH or NADPH reduced MMb and reduction was enhanced by presence of flavins. They found nonenzymatic reduction was more efficient than enzymatic reduction and that the addition of ATP, ADP or AMP did not affect reduction.

Al-Shaibani et al. (1977) reported the purification of MMb reductase from tuna. The enzyme was extracted by water and purified by Sephadex G-25 gel filtration. Purified enzyme had greater activity than crude extract. MMb reductase was inhibited by the addition of Cu^{++}.

Hagler et al. (1979) reported the isolation of a MMb reductase from bovine heart which was highly NADH-dependent with no reduction being apparent in the absence of NADH. In contrast to Brown and Snyder (1969) and Saleh and Watts (1968), Hagler et al. (1979) reported the

addition of NAD or NADP did not result in MMb reduction. Using a highly purified substrate, they found no non-enzymatic reduction. Like Brown and Snyder (1969), Hagler et al. (1979) found flavins improved MMb reduction. Enzyme activity was found to be proportional to enzyme concentration and no enzyme activity occurred when enzyme that had been treated to 50°C was added. Enzyme activity had an optimum pH of 6.5 and increased up to 37°C. From 37°C to 50°C, activity decreased until no activity was apparent after 50°C.

Oxygen Consumption by Meat Tissue

Oxygen consumption by tissue can indicate enzyme activity. Manometric (Warburg apparatus) and polarographic (oxygen electrodes) techniques have been the primary tools used to measure oxygen consumption of tissue and mitochrondrial respiration.

George and Stratmann (1952a) used the Warburg apparatus to measure the oxygen consumption during Mb oxidation. Grant (1955) studied postmortem muscle enzyme activity by using the Warburg apparatus. Succinic dehydrogenase, -glycerophosphate dehydrogenase, and cytochrome oxidase were active enzymes. By using the Warburg, Urbin and Wilson (1961) found the oxygen requirements of tissue decrease with decreasing temperature, due to decreased demand for oxygen by enzymes. Oxygen is used in meat for:

1. oxygenation of Mb, 2. tissue absorption of oxygen, 3. enzymatic activity, and 4. autoxidation of Mb and fats (Urbin and Wilson, 1958).

Oxygen consumption of bovine muscle is pH and temperature dependent (Urbin and Wilson, 1961). As pH and temperature decrease, so does oxygen consumption. Atkinson et al. (1969), measuring oxygen consumption of lamb SM muscle by manometric technique, found oxygen consumption and NAD concentration decreased with time postmortem. When adding 1 to 4 micromoles of NADH/ml of Ringers solution (3 ml), a 4-fold increase in oxygen consumption was observed for the largest addition.

DeVore and Solberg (1974) manometrically measured oxygen uptake in the SM muscle at 5°C at 5 to 7 days postmortem. Solutions were perfused into the entire muscle by using a stitch pump needle in an artery. Oxygen uptake by heme pigments was linear up to 30 hr and was accountable for approximately 50% of the total oxygen consumed. Oxygen uptake by heme pigments remained constant as total consumption decreased. Decay in respiratory oxygen consumption was credited to substrate depletion or enzyme degradation.

In studying oxygen consumption of pre-rigor muscle, Bendall (1972) found that the higher the temperature, the greater the oxygen consumption and that minces consumed oxygen faster than muscle strips. The latter behavior was thought to be due to cellular and tissue damage. Muscle

(primarily SM) was excised 10 min postmortem and prepared for study. Oxygen consumption was species dependent and membrane permeability seemed to play an important part in pre-rigor oxygen consumption rate. Beef tissue had the greatest OCR with rabbit, sheep and pigeon tissue being lower and about the same. ATP turn-over rate was the limiting factor in oxygen consumption rate except below 15°C.

Bendall and Taylor (1972) found mitochondrial respiration was the limiting factor in post-rigor oxygen consumption. In contrast to DeVore and Solberg's (1974) findings, Bendall and Taylor (1972) reported that oxygen consumption remained constant with declining NAD content. In post-rigor muscle, the degree of comminution had no effect on oxygen consumption rate. When carnosine base was added to raise pH to 7.2, muscle OCR increased as did mitochondrial rate. OCR exponentially declined during storage and was highly temperature dependent.

In 1953, Bulbring used polarographic techniques to measure the OCR of guinea-pig taenia coli muscle. Clark et al. (1953) proposed the use of a stretched cellophane membrane on oxygen electrodes to eliminate interference with probe signals caused by stirring and protein deposition on the electrode. The covered electrode response to temperature was linear and membrane stretch was important to sensitivity and equilibration time.

Brown and Mebine (1969) used a YSI oxygen electrode

to measure oxygen consumption of MbO and oxygen evolution upon oxidation of MbO. They reported .75 moles of oxygen is evolved upon oxidation of 1 mole MbO. Using a Clark electrode for oxygen consumption analysis, Ashmore et al. (1971) reported that pre-rigor muscle, dark cutting beef and normal beef tissue did not consume oxygen differently.

Oxygen consumption at 20°C is apparently not related to discoloration of frozen meat (Schafer, 1972). A YSI Clark electrode was used to measure oxygen consumption of bovine muscle, but the correlation between the amount of oxygen consumed in 30 min by LD muscle and %R630 nm was -.15. Solberg (1970) reported an oxygen consumption rate for fresh beef at 4°C of .05 microliters/ cm^2/min during the first three hours. OCR fell after three hours.

LeFevre et al. (1970) reviewed problems involved with using oxygen electrodes. Electrodes deteriorate due to AgCl deposits on the silver anode. Diffusion of gas into tissue and fluids can give erroneous results until an equilibrium is achieved. To avoid diffusion problems, tissue pieces should be small (.5 mm or less) so equilibrium can be reached in a negligible time. Air saturated buffers should probably be used rather than pure oxygen saturated buffers, because electrodes will last longer since AgCl crystals will not deposit on the silver anode as rapidly with air saturated buffers. But, oxygen gradients are smaller in air saturated systems and the

tissue may not have enough oxygen to prevent anoxia or tissue damage. Therefore, air systems may not always be sufficient.

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Chapter III

Effect of Electrical Stimulation on Myoglobin Properties of Bovine Longissimus Muscle

Introduction

Electrical stimulation improved longissimus (LD) muscle color of beef carcasses at the time of ribbing (Eikelenboom et al., 1981; Davis et al., 1981; Salm et al., 1981) and under retail display conditions (Hall et al., 1980). Others have found, however, no LD muscle color differences between electrically stimulated (ES) and control (C) carcasses at the time of ribbing (Savell et al., 1979; Grusby et al., 1976) or between ES and C steaks during display (Claus, 1982). Orcutt et al. (1981) reported that longissimus muscle from ES carcasses was lighter and redder than C muscle; however, no difference were found in the proportion of oxymyoglobin on steak surfaces. They suggested that color differences may be due to deeper oxygen penetration and/or greater light reflection from the meat surface. However, Tang and Henrickson (1980) reported a greater proportion of oxymyoglobin as determined by electrophoresis in ES muscle than C muscle. The objective of this research was to study the effects of carcass ES on color mechanisms and myoglobin properties of beef longissimus muscle.

Materials and Methods

Source of Materials

Forty Angus bulls, half of which were implanted near birth with Ralgro (36mg) and re-implanted about every 106 days, were fed (minimum of 196 days) a 75% concentrate diet and slaughtered at either 454 or 499 kg. The average USDA carcass quality grade was $Good^{75}$. Carcasses were split and alternating left and right sides were ES 45 min postmortem for 2 min using 420V, 60Hz, .68 sec on and .32 sec off, with approximately 1 amp delivered through the side. The other side served as a control. A series of 2.5 cm thick LD steaks were removed from the shortloin (1 at 24 hr and the others at 48 hr postmortem) for use in the color studies. One steak removed at 48 hr postmortem was divided transversely into central, medial, and lateral portions.

Display Color Stability Study

A steak removed at 48 hr postmortem was wrapped in polyvinylchloride (PVC) film and displayed for 7 days at $3^{o}C$ under 1076 lux of continuous Natural fluorescent lighting. Samples were bloomed in the dark for 1 hr before measuring percentage reflectance at selected wavelengths and Hunter L^{*}, a^{*}, and b^{*} (CIE) values of Illuminant C with a D54 Hunterlab spectrophotometer.

A five member color panel evaluated LD steaks during

display for overall lean color using the scale of 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 (Kropf et al., 1971). Steaks were evaluated visually and spectrophotometrically at 0, 1, 3, 5, and 7 days of display.

Blooming Studies

A steak removed immediately after ribbing (24 hr postmortem) was placed in an oxygen impermeable bag for transport to the laboratory. The steak was re-faced to expose a new surface and then was wrapped immediately in PVC film. Percentage reflectance at selected wavelengths and L^*, a^* and b^* values were measured spectrophotometrically within 15 sec after facing (time 0) and at 1, 2, 3, 4, 5, 8, 15, and 30 min post-facing. Samples were on the spectrophotometer approximately 10 sec during each scan and then they were stored in a display case at $3^{o}C$. A color panel scored samples that were under display lighting conditions at 1 hr post-facing.

The lateral one-third portion of a steak removed at 48 hr postmortem was vacuum packaged immediately after fabrication in 3 mil Saran-coated surlyn barrier film (<1 cc of oxygen/645 cm^2/24 hr at $23^{o}C$ and $0^{o}Rh$), and stored in the dark at $6^{o}C$. After 4 days of storage, the samples were faced, bloomed, and spectrophotometrically and visually evaluated as described for the steaks removed

and bloomed at 24 hr postmortem.

Metmyoglobin Reducing Activity

The central and medial one-third portions of a steak removed 48 hr postmortem were vacuum packaged immediately after removal, stored in the dark at 6°C and were used to study metmyoglobin reducing activity (MRA) by either of two methods.

At 10 days postmortem, the central one-third portion was removed from the package, submerged in 1% potassium ferricyanide for 30 sec to induce metmyoglobin (MMb) formation then the excess solution was blotted. Samples were left unpackaged at 27°C for 30 min and then re-packaged in oxygen impermeable film. Anaerobic reduction of MMb at 27°C was followed spectrophotometrically at 30, 60, 90, 120, 180, 240, and 360 min after chemical oxidation. Percentage reflectance of samples was read before oxidation to establish the initial percentage of reduced myoglobin (Mb).

The medial one-third portion of the steak was stored until 15 days postmortem, removed from the packaging film and covered with PVC film. Samples were placed in an anaerobic incubator, the incubator was evacuated and then flushed with a mixture of 1% oxygen and 99% nitrogen gas (repeated twice) to induce MMb formation (Ledward, 1972). The incubator was stored in the dark at 6°C for 26 hr. Samples were removed (0 time) and immediately scanned

spectrophotometrically. During sample removal, the chamber was continuously flushed with the 1% oxygen gas mixture. After all samples were scanned initially, samples were stored in the dark at 6°C and aerobic reduction of MMb was measured at 2, 4, 6, 8, 10, 12, and 24 hr after removal from the 1% oxygen atmosphere.

Spectrophotometric and Pigment Calculations

K/S value ratios of 474/525 nm and 572/525 nm were calculated to determine percentage MMb and percentage Mb, respectively. Oxymyoglobin (MbO) was calculated by difference. Constants used in pigment calculations determined for our spectrophotometer and packaging films were: .89 and .94 for 100% Mb, .40 and .51 for 0% Mb, 1.82 and 1.45 for 100% MMb, and .62 and .51 for 0% MMb for the oxygen permeable and impermeable films, respectively. Percentage reflectance differences at 630 nm minus 580 nm (%R630-%R580), were used to indicate differences in redness; the larger the difference, the brighter red the sample.

Total Pigment Analysis

Total pigment concentration was determined using Hornsey's (1956) acidified acetone procedure and ppm haematin was converted to mg/g wet wt using the .026 conversion factor of Franke and Solberg (1971). Pigments were extracted in the dark for 30 min, and preliminary

work indicated that the addition of .5% DL cysteine hydrochloride for color stability (DeVore and Solberg, 1974) was not necessary.

Statistical Analysis

Data were analyzed using analysis of variance procedures and means were separated using Duncan's Multiple Range test (SAS Institute, Inc., 1979). No interactions between cattle management system and stimulation treatment were found; therefore, the data were pooled and analyzed for electrical stimulation effects.

Results

Total Pigment Concentration

No differences ($P>.05$) were found between ES and C muscle for total pigment concentration (Table 4).

Display Color Stability

Steaks from ES carcasses had brighter red ($P<.05$) visual color scores than C steaks initially and at 1, 3, and 5 days of display (Table 5), but color differences were not apparent on day 7. Reflectance measurements also indicated that ES samples were both brighter ($P<.05$) red (larger differences at %R630-%R580 nm) and lighter ($P<.05$) in color (larger Hunter L^* values) than C samples at most evaluation times. No consistent differences ($P>.05$)

Table 4-Means for total pigment concentration in control (C) and electrically stimulated (ES) bovine longissimus steaks.

Treatment	Total pigment concentration mg/g wet tissue
C	5.3[a]
ES	5.6[a]

[a] Means having a different superscript are different ($P<.05$).

Table 5-Means for visual color, spectrophotometric measurements, and myoglobin forms for displayed control (C) and electrically stimulated (ES) bovine longissimus steaks

Display time (days)	Treatment	Visual panel score[a]	R630-R580	Hunter (CIE) values			Myoglobin forms[b]		
				L^*	a^*	b^*	%Mb	%MbO	%MMb
0	C	2.5^c	21.1^d	38.6^d	34.3^c	20.6^c	8.9^c	83.3^c	7.8^c
	ES	2.0^d	24.1^c	41.4^c	32.8^d	21.6^c	5.3^d	85.0^c	9.7^c
1	C	2.7^c	21.1^d	40.5^d	32.6^d	21.6^c	1.4^c	85.5^c	13.1^c
	ES	2.2^d	23.8^c	43.2^c	33.8^c	22.5^c	0.7^c	86.6^c	12.7^c
3	C	2.8^c	20.9^d	40.4^d	33.4^c	22.0^c	1.1^c	76.8^c	22.1^c
	ES	2.5^d	22.7^c	42.4^c	34.0^c	22.4^c	2.3^c	77.4^c	20.3^c
5	C	3.1^c	20.2^d	39.6^c	34.2^c	23.5^c	6.8^c	74.1^c	19.1^c
	ES	2.8^d	21.6^c	41.0^c	34.2^c	23.7^c	6.1^c	78.4^c	15.5^c
7	C	3.2^c	19.3^c	40.4^d	31.5^c	21.2^c	4.6^c	67.6^c	27.8^c
	ES	3.0^c	20.1^c	42.6^c	31.2^c	20.8^c	3.1^d	69.8^c	27.1^c

[a] 1 = very bright red, 2 = bright red, 3 = slightly dark red or brown, 4 = dark red or brown, 5 = extremely dark red or brown.

[b] Mb = reduced myoglobin, MbO = oxymyoglobin, MMb = metmyoglobin.

[c,d] Means for each time and within each column having different superscripts are different ($P<.05$).

were found between ES and C samples for Hunter a^* and b^* values and for differences in percentages of surface Mb, MbO, or MMb.

Blooming Studies

ES samples bloomed at either 24 hr (Table 6) or 6 days (Table 7) postmortem had brighter red ($P<.05$) visual color scores, greater ($P<.05$) %R630-%R580 nm differences, and larger ($P<.05$) Hunter L^* values than C samples at all evaluation times during blooming. No differences ($P>.05$) between ES and C samples bloomed at 24 hr postmortem were observed for Hunter a^* and b^* values, or for percentages of surface Mb (except at 5 and 30 min), MbO, and MMb. But, ES samples bloomed at 6 days postmortem had smaller $P<.05$) Hunter a^* values at 1 and 8 min, larger ($P<.05$) Hunter b^* values at 0, 2, 3, 4, and 5 min, less ($P<.05$) Mb at 0, 2, 3, 4, 5, 8, 15, and 30 min, more ($P<.05$) MbO at 2, 3, 4, 5, and 8 min, and more ($P<.05$) MMb at 2, 4, 5, and 8 min than C samples.

Table 8 presents a comparison of means from samples bloomed at 1 or 6 days postmortem. Visual color scores were similar for samples bloomed at 1 and 6 days postmortem, but samples bloomed at 6 days postmortem had greater ($P<.05$) differences at %R630-%R580nm, and had larger ($P<.05$) Hunter L^*, a^*, and b^* values than samples bloomed at 1 day postmortem. Six day old samples had considerably less ($P<.05$) surface Mb and more ($P<.05$)

Table 6-Means for visual color, spectrophotometric measurements, and myoglobin forms for control (C) and electrically stimulated (ES) bovine longissimus steaks bloomed at 24 hr postmortem

Time in air	Treatment	Visual panel score[a]	R630-R580	Hunter (CIE) values			Myoglobin forms[b]		
				L*	a*	b*	%Mb	%MbO	%MMb
0	C	-	15.8^{d}	33.8^{d}	28.6^{c}	10.6^{c}	76.2^{c}	20.1^{c}	3.7^{c}
	ES	-	17.1^{c}	35.9^{c}	25.6^{c}	10.6^{c}	73.8^{c}	17.5^{c}	8.7^{c}
1	C	-	16.4^{d}	34.6^{d}	28.7^{c}	11.3^{c}	68.3^{c}	25.7^{c}	6.0^{c}
	ES	-	17.5^{c}	35.9^{c}	29.2^{c}	11.6^{c}	68.1^{c}	25.4^{c}	6.4^{c}
2	C	-	16.5^{d}	34.8^{d}	28.9^{c}	12.0^{c}	63.7^{c}	31.1^{c}	5.2^{c}
	ES	-	17.8^{c}	36.2^{c}	28.6^{c}	12.1^{c}	62.7^{c}	31.1^{c}	6.1^{c}
3	C	-	16.8^{d}	34.7^{d}	29.2^{c}	12.5^{c}	60.8^{c}	36.7^{c}	2.6^{c}
	ES	-	18.0^{c}	36.3^{c}	29.6^{c}	12.7^{c}	59.1^{c}	36.0^{c}	4.9^{c}
4	C	-	16.7^{d}	34.6^{d}	29.2^{c}	12.7^{c}	58.1^{c}	37.7^{c}	4.2^{c}
	ES	-	18.0^{c}	36.5^{c}	29.4^{c}	12.7^{c}	56.5^{c}	38.0^{c}	5.5^{c}
5	C	-	16.9^{d}	36.2^{d}	29.9^{c}	13.2^{c}	58.1^{c}	37.4^{c}	4.5^{c}
	ES	-	18.3^{c}	36.6^{c}	29.5^{c}	13.0^{c}	54.1^{d}	39.3^{c}	6.6^{c}
8	C	-	17.1^{d}	34.9^{d}	29.6^{c}	13.4^{c}	52.8^{c}	44.1^{c}	3.1^{c}
	ES	-	18.4^{c}	36.7^{c}	29.8^{c}	13.6^{c}	50.9^{c}	44.3^{c}	4.8^{c}
15	C	-	17.0^{d}	35.6^{d}	28.8^{c}	13.1^{c}	47.5^{c}	42.6^{c}	9.9^{c}
	ES	-	18.4^{c}	37.4^{c}	29.2^{c}	13.3^{c}	44.5^{c}	43.2^{c}	12.3^{c}
30	C	2.5^{c}	17.3^{d}	36.5^{d}	28.5^{c}	13.4^{c}	39.4^{c}	46.6^{c}	14.1^{c}
	ES	2.0^{d}	19.3^{c}	38.5^{c}	28.4^{c}	13.5^{c}	34.9^{d}	47.4^{c}	17.7^{c}

[a] 1 = very bright red, 2 = bright red, 3 = slightly dark red or brown, 4 = dark red or brown, 5 = extremely dark red or brown.

[b] Mb = reduced myoglobin, MbO = oxymyoglobin, MMb = metmyoglobin.

[c,d] Means for each time and within each column having different superscripts are different ($P<.05$).

Table 7-Means for visual color, spectrophotometric measurements, and myoglobin forms for control (C) and electrically stimulated (ES) bovine longissimus steaks bloomed at 6 days postmortem

Time in air	Treatment	Visual color score[a]	R630-R580	Hunter (CIE) values			Myoglobin forms[b]		
				L*	a*	b*	%Mb	%MbO	%MMb
0	C	-	18.1[d]	37.7[d]	28.4[c]	10.8[d]	74.0[c]	15.7[c]	10.3[c]
	ES	-	19.8[c]	40.0[c]	28.3[c]	11.3[c]	70.2[d]	17.7[c]	12.1[c]
1	C	-	18.5[d]	37.4[d]	29.1[c]	11.8[c]	66.7[c]	24.3[c]	9.0[c]
	ES	-	20.0[c]	40.7[c]	28.3[d]	12.1[c]	61.2[c]	26.9[c]	11.9[c]
2	C	-	18.6[d]	38.1[d]	28.8[c]	12.3[d]	58.5[c]	29.3[d]	12.1[d]
	ES	-	20.4[c]	41.1[c]	29.3[c]	12.8[c]	51.9[d]	31.9[c]	16.2[c]
3	C	-	18.9[d]	38.3[d]	28.9[c]	12.8[d]	53.1[c]	34.0[d]	12.9[c]
	ES	-	20.6[c]	41.0[c]	28.8[c]	13.3[c]	47.5[d]	36.5[c]	16.0[c]
4	C	-	19.1[d]	38.3[d]	29.2[c]	13.3[d]	49.3[c]	37.9[d]	12.8[d]
	ES	-	20.8[c]	40.8[c]	29.0[c]	13.8[c]	43.4[d]	40.8[c]	15.8[c]
5	C	-	19.2[d]	38.4[d]	29.2[c]	13.4[d]	45.7[c]	40.5[d]	13.8[d]
	ES	-	21.0[c]	41.5[c]	28.9[c]	13.9[c]	38.5[d]	43.5[c]	18.0[c]
8	C	-	19.2[d]	38.3[d]	30.0[d]	14.7[c]	41.6[c]	46.8[d]	11.3[d]
	ES	-	21.5[c]	41.4[c]	29.7[c]	15.2[c]	31.8[d]	51.7[c]	16.5[c]
15	C	-	20.4[d]	39.0[d]	30.5[c]	16.0[c]	29.1[c]	55.9[c]	14.9[c]
	ES	-	22.4[c]	42.4[c]	30.0[c]	16.2[c]	20.0[d]	60.8[c]	19.2[c]
30	C	2.4[c]	21.6[d]	39.4[d]	31.8[c]	18.0[c]	18.1[c]	68.2[c]	13.7[c]
	ES	2.0[d]	23.7[c]	42.8[c]	31.6[c]	18.2[c]	10.7[d]	72.1[c]	17.2[c]

[a] 1 = very bright red, 2 = bright red, 3 = slightly dark red or brown, 4 = dark red or brown, 5 = extremely dark red or brown.

[b] Mb = reduced myoglobin, MbO = oxymyoglobin, MMb = metmyoglobin.

[c,d] Means for each time and within each column having different superscripts are different ($P<.05$).

Table 8-Means comparing control (C) and electrically stimulated (ES) bovine longissimus samples bloomed at either 1 or 6 days postmortem

Measurement[a]	Treatment	Bloom time	
		1 day	6 days
Visual color score	C	2.5[b]	2.4[b]
	ES	2.0[c]	2.0[c]
R630-R580	C	17.3[e]	21.6[c]
	ES	19.3[d]	23.7[b]
Hunter L*	C	36.5[d]	39.4[c]
	ES	38.5[c]	42.8[b]
Hunter a*	C	28.5[c]	31.8[b]
	ES	28.4[c]	31.6[b]
Hunter b*	C	13.4[c]	18.0[b]
	ES	13.5[c]	18.2[b]
Reduced myoglobin, %	C	39.4[b]	18.1[d]
	ES	34.9[c]	10.7[e]
Oxymyoglobin, %	C	46.6[d]	68.2[c]
	ES	47.4[d]	72.1[b]
Metmyoglobin, %	C	14.1[b]	13.7[b]
	ES	17.7[b]	17.2[b]

[a] Samples evaluated after blooming for 30 min.

[b,c,d,e] Means for the same measurement with different superscripts are different ($P<.05$).

surface MbO than samples bloomed at 1 day postmortem, but percentages of surface MMb were similar.

Metmyoglobin Reducing Activity

Percentage surface MMb of ES and C samples was not different ($P>.05$) initially or 30 min after oxidation with potassium ferricyanide (Figure 1, Appendix E). C muscle had less ($P<.05$) surface MMb at 1, 1.5, 2, 2.5, 3, and 3.5 hr after oxidation during anaerobic reduction. No differences ($P>.05$) between ES and C samples for percentage surface MMb occurred after reducing for 4 and 5 hr.

ES samples formed more ($P<.05$) surface MMb (0 time) in 1% oxygen than C samples (Figure 2, Appendix F) and ES samples had more ($P<.05$) surface MMb at 2, 4, 10, and 12 hr of aerobic reduction.

Figure 3 presents the percentage of MMb reduced over time under aerobic and anaerobic conditions. C samples in an anaerobic environment had reduced a greater ($P<.05$) percentage of MMb at 1, 1.5, 2, 2.5, 3, and 3.5 hr than ES samples. But, the percentage of MMb reduced aerobically was not different ($P>.05$) for ES and C samples at any time.

Discussion

Total Pigment Concentration

These data like those of Tang and Henrickson (1980)

Figure 1-Mean percentages (±SE) of surface metmyoglobin on control and electrically stimulated bovine longissimus samples initially (I) and during anaerobic reduction. Metmyoglobin was formed by oxidation with potassium ferricyanide.

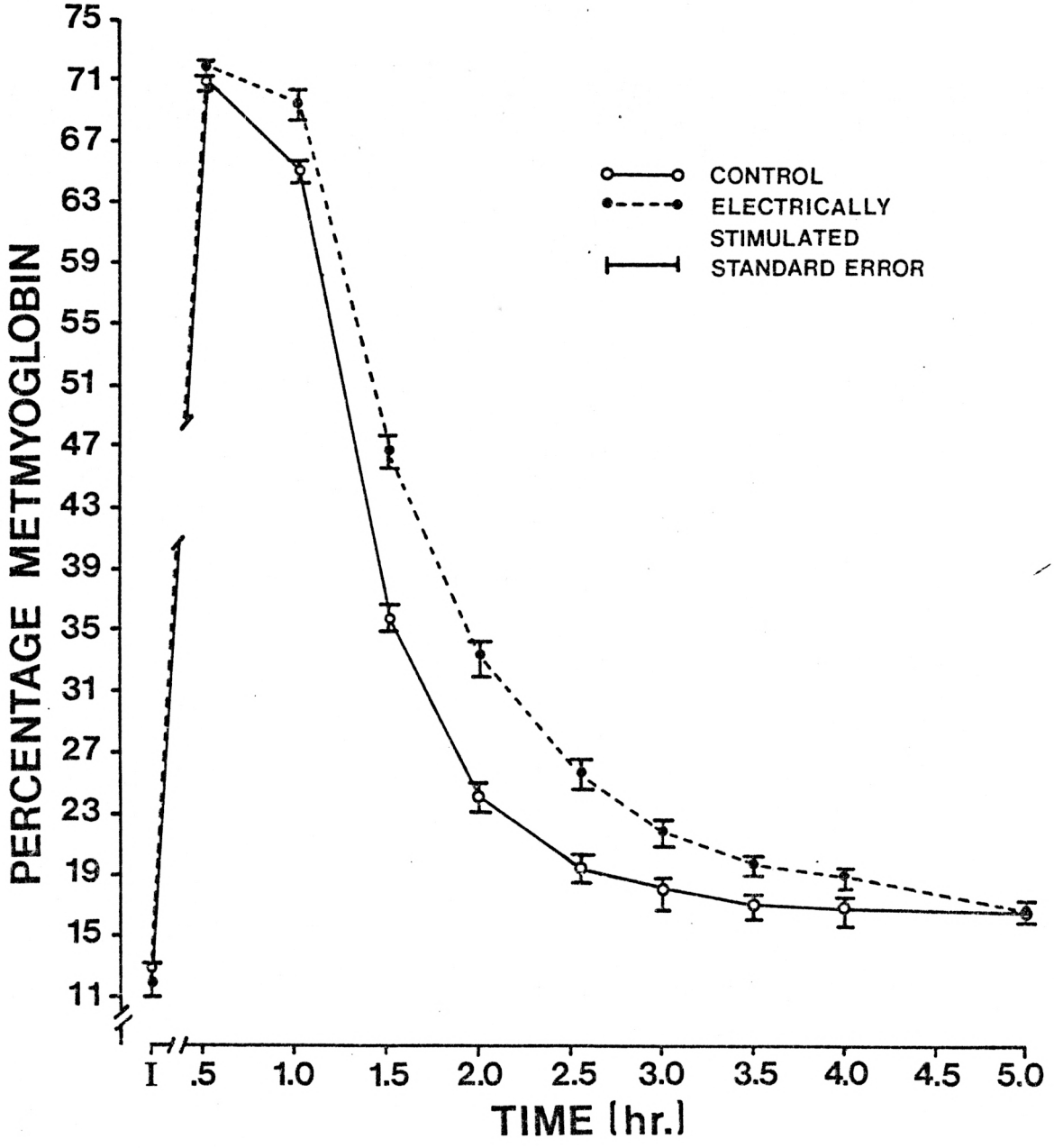

PERCENTAGE METMYOGLOBIN
75
71
67
63
59
55
51
47
43
39
35
31
27
23
19
15
11
CONTROL
ELECTRICALLY STIMULATED
STANDARD ERROR
I
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
TIME (hr.)

Figure 2-Mean percentages (±SE) of surface metmyoglobin on control and electrically stimulated bovine longissimus samples during aerobic reduction. Metmyoglobin was formed by oxidation in a 1% oxygen atmosphere.

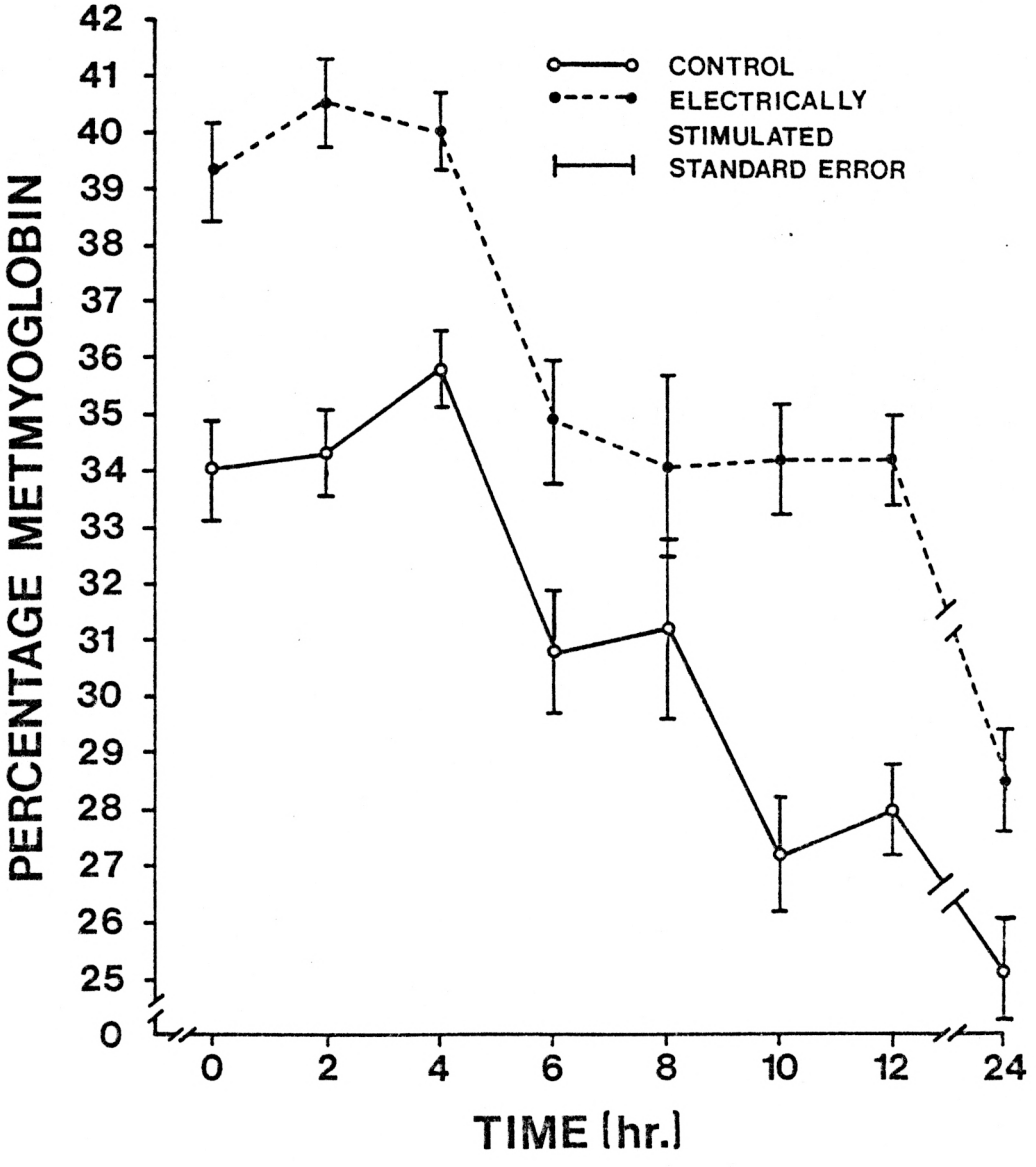
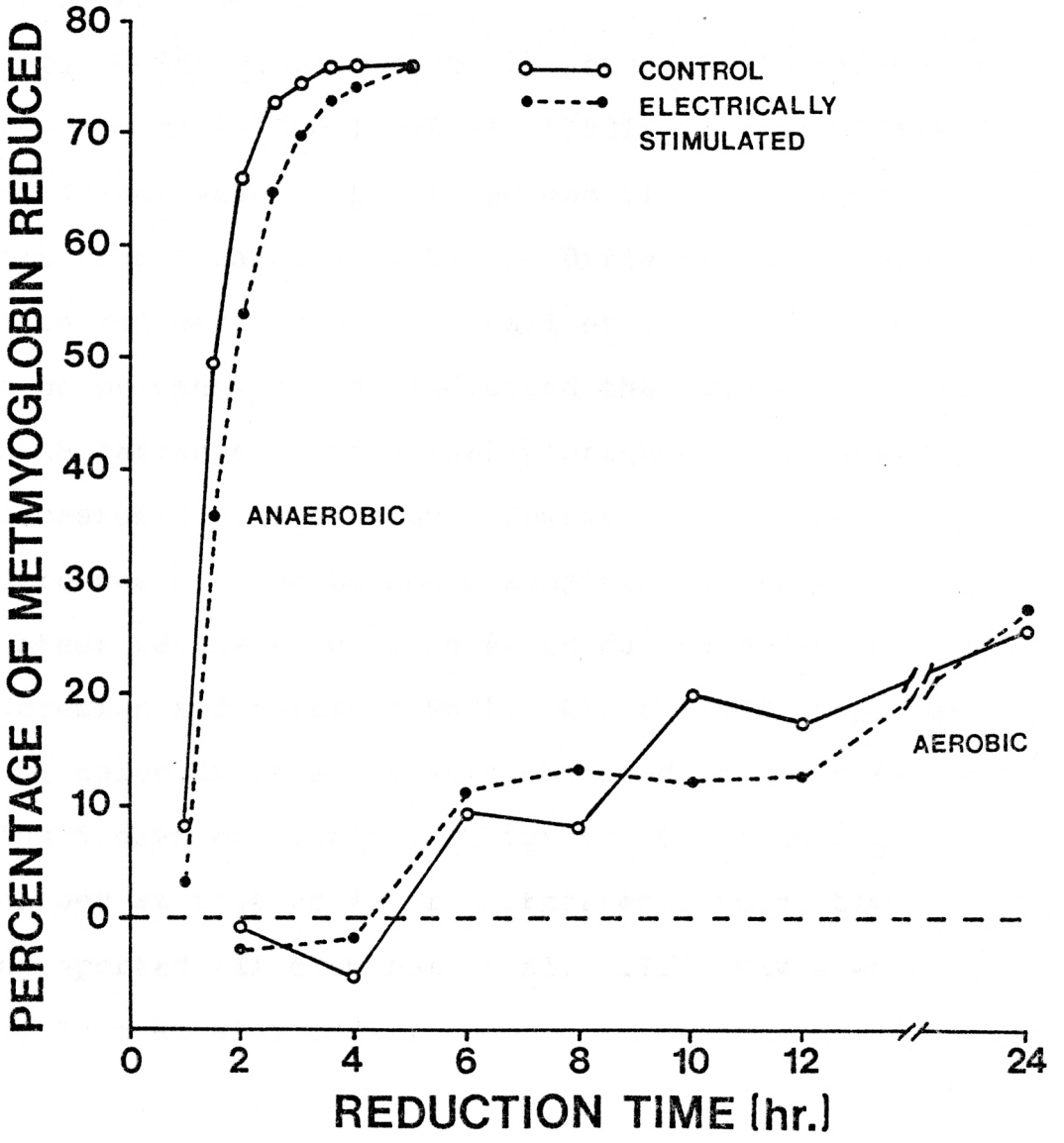

PERCENTAGE METMYOGLOBIN
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
0
0
2
4
6
8
10
12
24
TIME (hr.)
CONTROL
ELECTRICALLY STIMULATED
STANDARD ERROR

Figure 3-Percentage of metmyoglobin reduced by control and electrically stimulated bovine longissimus samples during anaerobic and aerobic reduction.


PERCENTAGE OF METMYOGLOBIN REDUCED
80
70
60
50
40
30
20
10
0
CONTROL
ELECTRICALLY
STIMULATED
ANAEROBIC
AEROBIC
0
2
4
6
8
10
12
24
REDUCTION TIME (hr.)

indicate no differences in total pigment concentration of ES and C muscle. Because no differences in total pigment were found in this study, color differences cannot be attributed to differences in pigment quantity.

Color Display Stability

Our color display stability results are similar to those reportd by Orcutt et al. (1981) in that steaks from ES carcasses were brighter red and lighter colored than steaks from C carcasses, but no differences in percentage surface MbO were observed. Hall et al. (1980) reported that color panel scores indicated that adductor steaks from ES carcasses were visually brighter than steaks from C carcasses during display. However, no muscle color differences between ES and C samples from bull, heifer, and steer carcasses were observed during 4 days of display by Jeremiah and Martin (1980). Claus (1982) reported no visual color differences were observed in C and ES samples during 5 days of display. Brighter LD muscle color in ES carcasses at a 24 or 48 hr postmortem ribbing time has been reported (Eikelenboom et al., 1981; Davis et al., 1981; Salm et al., 1981).

Blooming Studies

Although ES muscle was brighter red and lighter than C muscle during blooming at both 24 and 6 days postmortem, color differences cannot be attributed solely to different

proportions of myoglobin on steak surfaces. More significant differences were observed for percentages of Mb, MbO, and MMb on both C and ES samples bloomed at 6 days postmortem than at 24 hr postmortem, an effect attributed to aging. The extent of change from 0 to 30 min of bloom time in %R630-%R580 nm and Hunter L^* values at both 24 hr and 6 days postmortem was essentially the same for C and ES samples. Apparently, the effects of electrical stimulation on muscle color involves other factors besides pigment concentration and changes in myoglobin forms.

Cross et al. (1979) studied combinations of cloth shrouding and PVC film wrapping of C and ES carcasses. Shrouded ES carcasses had better muscle color than shrouded C carcasses, but no color differences were observed between shrouded ES carcasses, PVC wrapped ES carcasses, and shrouded-PVC wrapped C carcasses. However, C shrouded-PVC wrapped carcasses had better muscle color scores than C shrouded sides. Their results suggest that a possible slower chilling rate of shrouded-PVC film wrapped C carcasses resulted in muscle color similar to effects of ES on beef muscle color. Kastner et al. (1980) displayed hot-boned electrically stimulated samples and found no color advantage over displayed C samples, but hot-boned cuts chill at a faster rate than cuts chilled in an intact carcass. A faster chilling rate may be negating the total effects of ES on muscle color, but the combination of hot-boning and ES does prevent the darkening of

muscle color obseved in hot-boning alone (Taylor et al., 1981; Claus, 1982).

Savell et al. (1978) observed more structural damage in ES beef muscle. This tissue disruption may result in a more loose muscle structure that allows deeper oxygen penetration, thus resulting in a thicker MbO layer. The looser structure may also cause more light scatter and consequently a lighter muscle appearance without changing the percentage of surface MbO. George et al. (1980) compared structural damage in ES bovine muscle to that seen in PSE porcine muscle. Dutson et al. (1980) reported that ES ovine muscle had more released lysosomal enzymes; however, a greater percentage of these enzymes were degraded due to the combination of higher muscle temperature and a lower pH of ES muscle. Ashmore et al. (1972) reported that enzymes of dark cutting beef which has a high pH and a tight muscle structure were very competitive for available oxygen. Thus, less oxygen would be available for Mb oxygenation. Although enzyme activity or structural damage were not measured in this study, the spectrophotometric measurements indicate ES muscle was lighter in color and reflected more light from its surface. If enzymes important to muscle color are also degraded due to temperature-pH conditions in ES carcasses, enzyme activity may be lower in ES muscle which would allow deeper oxygen penetration into ES muscle.

Metmyoglobin Reducing Activity

The two metmyoglobin reducing activity methods appear to give conflicting results. During anaerobic reduction, C samples had a more active MMb reducing mechanism than ES samples. However, under aerobic conditions, ES samples formed more MMb, but the percentage reduced over time was not different than from C samples. These data support the suggestion by Giddings (1974) that there are separate enzymatic mechanisms for aerobic and anaerobic reduction.

If more lysosomal membranes are ruptured and more enzymes are denatured (Dutson et al., 1980) by electrical stimulation of carcasses, then possibly other subcellular organelle membranes such as mitochondrial membranes may also be effected by ES. Recent reviews (Giddings, 1974; Livingston and Brown, 1981) discuss the importance of mitochondrial activity to meat color, especially MRA. Watts et al. (1966) reported the addition of NAD to muscle samples could restore MRA and oxygen had to be limited for MRA to occur. They noted that electrons from NADH react preferentially with oxygen rather than with the reducing mechanism. Ledward (1971) reported rapid MRA depletion in the presence of oxygen and Stewart et al. (1965) reported little MRA occurred below 15^{o}C; therefore, the temperature conditions (6^{o}C) of the aerobic study may have been rate limiting for both C and ES samples.

Based upon this research, it was concluded that: 1. total pigment concentration did not effect meat color

differences between C and ES muscle, 2. color differences between C and ES muscle were not due to different surface proportions of Mb, MbO, or MMb, 3. ES muscle was lighter and reflected more light from its surface than C muscle, perhaps due to structural changes in ES muscle, and 4. under certain conditions ES muscle was more susceptible to MMb formation and had less MRA.

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Chapter IV

Interrelationships of Spectrophotometric Measurements and Their Relationships to Beef Longissimus Muscle Visual Color

Introduction

Attempts to replace visual color panels with spectrophotometric analysis (Ockerman and Cahill, 1969; Eagerman et al., 1977) have met limited success; however, some spectrophotometric measurements have been consistently good indicators of visual color. Strange et al. (1974) reported that Gardner a values, %R630-%R580 nm and ln(%R580/%R630) correlated best with visual color scores. Leising (1975) and Harrison et al. (1980) reported high correlations (-.82 and -.82, respectively) between visual color scores and %R630-%R580 nm.

Percentages of myoglobin forms on meat surfaces are important to visual color and these proportions can be determined by using ratios of reflectance at isobestic wavelengths (Hunt, 1980). Percentage reflectance values are converted to K/S values to increase linearity with pigment forms (Stewart et al., 1965). Percentages of reduced myoglobin (Mb) and metmyoglobin (MMb) can be calculated using ratios of 474/525 nm (Snyder and Armstrong, 1967) and 572/525 nm (Stewart et al., 1965), respectively. Oxymyoglobin (MbO) percentages can be

calculated by difference. This study correlates spectrophotometric measurements and visual color scores of beef longissimus muscle displayed for 7 days.

Materials and Methods

Forty Angus bulls, half of which were implanted near birth with Ralgro, were fed (minimum of 196 days) a 75% concentrate diet and slaughtered at either 454 or 499 kg. The average USDA carcass quality grade was Good[75]. Carcasses were split and alternating left and right sides were electrically stimulated at 45 min postmortem for 2 min using 420V, 60Hz, .68 sec on and .32 sec off, with approximately 1 amp delivered through the side. The other side served as a control. Longissimus steaks 2.5 cm in thickness were fabricated from the shortloin at 48 hr postmortem, wrapped in polyvinylchloride film and displayed under 1076 lux of continuous Natural fluorescent lighting at 3°C for 7 days.

Hunter L^*, a^*, and b^* values (CIE) of Illuminant C and percentage reflectance at 474, 525, 572, 580, and 630 nm were measured. Ratios of wavelengths and differences at 630-580 nm were calculated using both percentage reflectance values and their K/S equivalents (Judd and Wyszecki, 1963).

A five member color panel evaluated longissimus steaks during display for overall lean color using the scale of 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 (Kropf et al., 1971). Steaks were evaluated visually and spectrophotometrically at 0, 1, 3, 5, and 7 days of display.

Simple correlation coefficients (Snedecor and Cochran, 1967) were calculated for pooled color data since plots of control and electrically stimulated sample values did not indicate separate correlation analyses were necessary.

Results

Percentage reflectance (%R) ratios of 474/525 nm, 572/525 nm and 630/474 nm were highly ($P<.01$) intercorrelated, and all %R ratios were highly ($P<.01$) correlated with their respective K/S ratios (Table 9). Ratios of K/S values at 572/525 nm were negatively correlated with K/S value ratios of 474/525 nm (-.70) and 630/474 nm (-.78). The %R630-%R580 nm difference was not significantly correlated (-.01) to its corresponding K/S value difference. Correlations were low between the %R630-%R580 nm difference and the %R and K/S ratios, but the K/S difference at 630-580 nm correlated highly to both the %R and K/S ratios.

Correlations for Hunter L^* values with Hunter a^* and b^* values, and the a^*/b^* ratio were -.55, -.62 and .58, respectively. Hunter a^* values were highly correlated ($P<.01$) with Hunter b^* values (.94) and the

Table 9-Simple correlations[a] for spectrophotometric measurements[b] and visual color scores of beef longissimus steaks

Measurement	R474/R525	R572/R525	R630/R474	K474/K525	K572/K525	K630/K474	R630-R580	K630-K580	Hunter L*	Hunter a*	Hunter b*	a*/b*
R474/R525	-											
R572/R525	-.81	-										
R630/R474	.77	-.95	-									
K474/K525	-.99	.76	-.73	-								
K572/K525	.75	-.95	.92	-.70	-							
K630/K474	-.60	.87	-.88	.58	-.78	-						
R630-R580	-.06	-.35	.30	.03	.31	-.63	-					
K630-K580	-.86	.90	-.92	.80	-.92	.69	-.01	-				
Hunter L*	-.80	.67	-.69	.75	-.59	.47	.31	.77	-			
Hunter a*	.69	-.91	.92	-.67	.82	-.96	.51	-.76	-.55	-		
Hunter b*	.76	-.90	.92	-.73	.84	-.86	.32	-.83	-.62	.94	-	
a*/b*	-.69	.68	-.71	.66	-.62	.60	-.05	.70	.58	-.72	-.90	-
Visual color score	.32	.04	.05	-.29	-.03	.28	-.79	-.26	-.49	-.14	.06	-.28

[a] Correlations > .13 are significant at P<.01.

[b] R = percentage reflectance values, K = K/S values.

a^*/b^* ratio (-.72). Hunter b^* values were negatively correlated (-.90) with the a^*/b^* ratio.

Visual color scores correlated best with the %R630-%R580 nm difference (.79) and Hunter L^* values (-.49). All other correlations with visual color scores were low.

Discussion

Visual color scores provide information about acceptability of meat color and spectrophotometric measurements provide information about percentages of pigment forms on the meat surface and may indicate the redness of a meat sample, but knowing how spectrophotometric measurements relate to visual color scores is important.

K/S values are used to increase linearity with pigment forms (Stewart et al., 1965). But, ratios for Mb and MMb expressed in K/S units did not correlate any better with visual color scores than did their respective ratios expressed as percentage reflectance. The low correlations indicate that visual color scores are not dependent upon a single variable of either percentage Mb or MMb. Furthermore, visual color scores may not be linearly related to high percentages of surface MMb since Greene et al. (1971) reported that unacceptable visual color scores were related with 30 to 40% surface MMb. Therefore, a visual color response curve may plateau after unacceptable color is reached while the percentage of MMb

continues to increase. Even highly trained color panelists have a pre-conceived perception of the "ideal meat color" and rate samples accordingly (Eagerman et al., 1977). Fat color, non-uniformity of muscle color, marbling, lighting type and intensity, and panelists' pre-conception of "idea meat color" all may influence visual perception of meat color; whereas, a spectrophotometer only scans a portion of a sample and does not have the ability to change its response based on psychophysical perception.

The %R630-%R580 nm difference had the best correlation (-.79) with visual color scores. This agrees with Leising (1975), Harrison et al. (1980) and Strange et al. (1974) who reported correlations of -.82, -.82 and .86, respectively. The %R630-%R580 nm difference is apparently an indicator of how bright red a sample is and is based upon relative pigment amounts. The correlation (-.49) between Hunter L^* values and visual color scores was considerably higher than the correlation coefficient (.13) reported by Eagerman et al. (1978). This correlation indicates that color panel scores are affected by the lightness of a sample. The color panel scale could influence such a relationship because panelists may associate a lighter sample with a brighter red sample.

Of all spectrophotometric measurements, the %R630-%R580 nm difference correlated (-.79) best with visual color scores. The Hunter L^* value was significantly

correlated (-.47) with visual color score. As Leising (1975) also reported, conversion of %R values to K/S values for prediction of visual color scores is not necessary because conversion does not improve wavelength ratio correlation with visual color scores, but conversion to K/S values is necessary if knowledge of surface pigment forms is desired.

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APPENDICES

Appendix A

Metmyoglobin reducing activity method - 1% oxygen[a].

1. Remove a 2.5 cm thick steak 48 hr postmortem. Minimize exposure to oxygen and vacuum package in oxygen impermeable film immediately.

2. Store samples in the dark at 6°C for 13 additional days.

3. Remove sample. Cover surface with oxygen permeable film (to prevent dehydration) and place in an anaerobic incubator.

4. Evacuate incubator and flush with a 1% oxygen-99% nitrogen gas mixture. Repeat flushing procedure.

5. Control chamber temperature or store chamber in dark at 6°C for 26 hr.

6. Remove samples a few at a time to measure percent reflectance with a Hunterlab D54 spectrophotometer at wavelengths of 525 and 572 nm for calculating percentage metmyoglobin formed (0 time). Continuously flush chamber with 1% oxygen-99% nitrogen gas to maintain constant atmosphere.

7. Store samples in air and dark at 6°C for 24 hr for aerobic metmyoglobin reduction.

8. Repeat spectrophotometric readings at 2, 4, 6, 8, 10, 12, and 24 hr post-removal from chamber.

9. Use K/S ratios to determine percentage metmyoglobin present at each time and the percentage of metmyoglobin reduced over time. (Ledward determined %metmyoglobin formed after 24 hr in 1% oxygen and then determined the % metmyoglobin reduced after 24 hr of reduction time. He plotted %metmyoglobin reduced against %metmyoglobin formed on other samples after 7 days exposure to air.)

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[a] Modified procedure of Ledward, D.A. 1972. Metmyoglobin reduction and formation in beef during aerobic storage at 1°C. J. Food Sci. 37:634.

Appendix B

Metmyoglobin reducing activity method - 1% $K_3Fe(CN)_6$

1. Remove a 2.5 cm thick steak 48 hr postmortem. Minimize exposure to oxygen and vacuum package in oxygen impermeable film immediately.

2. Store samples in the dark at 6°C for 8 additional days. Measure percent reflectance with a D54 Hunterlab spectrophotometer at wavelengths of 474, 525, and 572 nm.

3. Remove sample from the packaging film. Dip in 1% $K_3Fe(CN)_6$ for 30 sec. Remove sample and blot excess ferricyanide solution. Expose samples to air for 30 min and vacuum package.

4. Immediately measure percent reflectance on the spectrophotometer (30 min post-treatment) at the specified wavelengths for calculating initial percentage metmyoglobin formed. Allow samples to anaerobically reduce metmyoglobin at 27 C° and determine percentage reflectance at 60, 90, 120, 150, 180, 210, 240, and 300 min post-oxidation.

5. Use K/S ratios to calculate percentage metmyoglobin and percentage reduced myoglobin at each time period. The percentage of metmyoglobin reduced can also be determined.

Appendix C

Reflectance constants for calculation of myoglobin forms for meat packaged in PVC or BIVAC oxygen-impermeable film.

1. Remove 2.5 cm thick steaks 48 hr postmortem. Vacuum package in oxygen impermeable film and store in the dark at 6°C until 9 days postmortem.

2. Transversely cut steaks in half. Place the lateral one-half in a bomb calorimeter chamber and submit the sample to a high partial pressure of oxygen for 5 to 10 min. Remove, wrap in PVC film and immediately measure percent reflectance spectophotometrically from 400 to 710 nm every 2 nm. Vacuum package samples and measure reflectance again.

3. Dip oxygenated samples in 1% $K_3Fe(CN)_6$ for 1 min, blot excess ferricyanide solution and wrap in PVC film. Allow to oxidize 12 to 24 hr. Scan as for oxygenated sample. Vacuum package and scan again.

4. Dip the medial one-half of the steak in 10% dithionite (NaS_2O_4), blot excess solution, vacuum package and allow pigment to reduce for 1 hr. Scan spectrophotometrically, then wrap in PVC film and scan again.

5. For each packaging film, plot mean percent reflectance versus wavelengths for each pigment form.

6. Calculate needed K/S ratio reflectance constants for calculation of percent pigment forms.

Appendix D

Total pigment determination[a]

1. Remove a 2.5 cm thick steak 48 hr postmortem, vacuum package, store in the dark at 6°C for 5 days, then freeze.

2. Dice frozen muscle (exterior fat and connective tissue excluded) and pulverize tissue in liquid nitrogen.

3. Store pulverized tissue at -80°C until used.

4. Weigh a 10 gr sample into a 250 ml beaker, add 10 ml solvent, stir until tissue is well dispersed and add 33 ml of solvent. Stir, cover with a watch glass and store in the dark for 30 min. During extraction, occasionally swirl beaker. Run duplicates of all samples.

   Solvent:
   40 parts acetone
   2 parts distilled water
   1 part concentrated HCl

5. Blank the spectrophotometer using a mixture of 43 ml solvent and 7 ml distilled water.

6. After extraction, filter through Whatman #2 filter paper and immediately read the optical density of the filtrate at 640 nm. Multiply reading by 680 to obtain ppm haematin.

7. Multiply ppm haematin by .026[b] to obtain mg total pigment/g wet tissue.

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[a] Method of Hornsey, H.C. 1956. The colour of cooked cured pork. I.-Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 7:534.

[b] Conversion factor is from Franke, W.C. and Solberg, M. 1971. Quantitative determination of metmyoglobin and total pigment in an intact meat sample using reflectance spectrophotometry. J. Food Sci. 36:515.

$$.026 = \frac{\text{Molecular weight of myoglobin (17000)}}{\text{Molecular weight of hematin (656000)}}$$

Appendix E

Table 10-Mean percentages of metmygglobin (MMb) and reduced myoglobin (Mb) before and after chemical oxidation and during anaerobic reduction in control (C) and electrically stimulated (ES) bovine longissimus steaks

Time (hr) after oxidation	Myoglobin form				Percentage of MMb reduced	
	percentage MMb		percentage Mb			
	C	ES	C	ES	C	ES
Before oxidation	12.9[a]	11.7[a]	86.3[a]	87.1[a]	-	-
.5	70.8[a]	71.8[a]	7.8[a]	6.9[a]	-	-
1.0	65.0[b]	69.6[a]	24.2[a]	20.2[b]	8.5[a]	3.2[b]
1.5	35.8[b]	46.4[a]	56.4[a]	45.1[b]	49.8[a]	35.6[b]
2.0	24.1[b]	33.1[a]	70.1[a]	58.3[b]	66.1[a]	54.1[a]
2.5	19.4[b]	25.5[a]	76.5[a]	66.9[a]	72.7[a]	64.6[b]
3.0	18.2[b]	21.9[a]	78.2[a]	73.4[b]	74.4[a]	69.6[b]
3.5	17.1[b]	19.6[a]	79.8[a]	75.7[b]	75.9[a]	72.8[b]
4.0	17.1[a]	18.7[a]	79.9[a]	77.2[b]	75.8[a]	74.0[a]
5.0	16.8[a]	16.7[a]	79.8[a]	79.0[a]	75.8[a]	76.1[a]

[a,b] C and ES means for each time and for the same variable with different superscripts are different ($P<.05$).

Appendix F

Table 11-Mean percentages of surface metmyoglobin (MMb) during aerobic reduction in control (C) and electrically stimulated (ES) bovine longissimus steaks

Time (hr) after removal from 1% oxygen atmosphere	MMb percentage		Percentage of MMb reduced over time	
	C	ES	C	ES
0	34.0^{b}	39.3^{a}	-	-
2	34.3^{b}	40.5^{a}	$-\ .9^{a}$	$-\ 3.1^{a}$
4	35.8^{b}	40.0^{a}	$-\ 5.3^{a}$	$-\ 1.8^{a}$
6	30.8^{a}	34.9^{a}	9.4^{a}	11.2^{a}
8	31.2^{a}	34.1^{a}	8.2^{a}	13.2^{a}
10	27.2^{b}	34.5^{a}	20.0^{a}	12.2^{a}
12	28.0^{b}	34.2^{a}	17.6^{a}	12.9^{a}
24	25.2^{a}	28.5^{a}	25.9^{a}	27.5^{a}

^{a,b} C and ES means for each time and for the same variable with different superscripts are different ($P<.05$).

MYOGLOBIN PROPERTIES OF ELECTRICALLY STIMULATED BOVINE LONGISSIMUS MUSCLE

by

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B.S., University of Missouri-Columbia, 1979

AN ABSTRACT OF A MASTER'S THESIS

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The purpose of this research was to study carcass electrical stimulation effects on color mechanisms and myoglobin properties of beef longissimus muscle.

Forty Angus bulls, half of which were implanted with Ralgro, were fed (minimum of 196 days) a 75% concentrate diet and slaughtered at either 454 or 499 kg. The average carcass USDA quality grade was Good^{75}. Carcasses were split and alternating left and right sides were electrically stimulated (ES) 45 min postmortem for 2 min using 420 V, 60 Hz, .68 sec on and .32 sec off, with approximately 1 amp delivered through the side. The other side served as a control (C). A series of 2.5 cm thick longissimus steaks were removed from the shortloins at either 24 or 48 hr postmortem for the color studies. No interactions between cattle management system and stimulation treatment were found; therefore, data were pooled and analyzed for electrical stimulation effects.

Total pigment concentration in ES and C muscles was not different ($P>.05$). ES samples displayed under 1076 lux of continuous Natural fluorescent lighting had brighter red visual color scores ($P<.05$) and larger differences ($P<.05$) in percentage reflectance (%R) at 630-580 nm (indicates a brighter red color) than C samples at 0, 1, 3, and 5 days of display. ES samples had larger Hunter L^* ($P<.05$) values than C samples at 0, 1, 3, 5, and 7 days of display. Generally, no differences ($P>.05$) were observed between ES and C groups for Hunter a^* and b^* values, and percentage

surface reduced myoglobin (Mb), oxymyoglobin (MbO), and metmyoglobin (MMb).

ES samples bloomed at either 24 hr or 6 days postmortem had brighter red visual color scores ($P<.05$), greater %R630-%R580 nm differences ($P<.05$) and larger Hunter L^* values ($P<.05$) than C samples at all evaluation times during blooming. No consistent differences ($P>.05$) were found in Hunter a^* and b^* values of ES and C samples bloomed at either 24 hr or 6 days postmortem. Percentages of surface Mb, MbO or MMb were not different ($P<.05$) for ES and C samples bloomed at 24 hr postmortem. ES samples bloomed at 6 days postmortem had less ($P>.05$) Mb, and generally more ($P<.05$) MbO at early blooming evaluation times and more ($P<.05$) MMb at later evaluation times than C samples.

Metmyoglobin reducing activity (MRA) of ES samples oxidized with potassium ferricyanide and reduced anaerobically was less ($P<.05$) than in C samples at all evaluation times except at 4 and 5 hr of reduction. MRA of ES and C samples oxidized in 1% oxygen and reduced in air was not different ($P>.05$). But, ES samples formed more ($P<.05$) MMb initially in 1% oxygen and had more MMb at most evaluation times during aerobic reduction.

Reflectance differences at 630-580 nm had the highest correlation (-.79) with visual color scores of all spectrophotometric measurements. Hunter L^* value was significantly correlated (-.47) with visual color score.

Ratios of selected wavelengths expressed as percentage reflectance correlated well with their respective ratios expressed as K/S values. Differences in percentage reflectance at 630-580 nm had a low correlation (-.01) with differences in K/S values at 630-580 nm.