THE EFFECT OF SUBJECTIVE MATURITY AND MARBLING ON PREPACKAGED BOVINE LONGISSIMUS DORSI MUSCLE COLOR AS MEASURED BY VISIBLE REFLECTANCE SPECTROPHOTOMETRY

by 6402

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B. S., Kansas State University, 1960

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

Food Science

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

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ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to Dr. D. H. Kropf, major professor, for his very sincere guidance and patience during this masters program. Appreciation is also expressed to Dr. H. J. Tuma for his efforts in design and coordination of the entire project. I am also indebted to Dr. A. D. Dayton, Department of Statistics and Computer Science, for his assistance in analysis of data.

Special thanks are extended to Dr. D. L. Grant and to R. C. Covington for their very able help in sample selection, fabrication, as well as technical support.

I know my father, Curtis D. Allen, and mother, Opal G. Allen, join me in expressing our keen appreciation to Kansas State University, its various colleges, departments, faculty, alumni, and many partisans for eternal conducement imparted to this family through the excellent academic processes administered.

I wish to thank Mrs. Linda Duhan for typing the many drafts of text and tables.

To his wife, Gayla Jo, the author expresses heartfelt praise for her support, understanding, enduring faith and subtle inducement during the long months of this work.

ORGANIZATION OF THE THESIS

This thesis is presented as a series of chapters. Chapter 1 is a general introduction to the entire thesis including a statement of the problem, reason for studying the topic and the specific purpose of the work. Chapter 2 includes a comprehensive review of literature pertinent to all topics and subjects included in subsequent chapters.

The three chapters following the general literature review each deal with an individual sub-unit of the total study and are placed in sequence as the study developed. Each of these chapters is written, with few exceptions, in the style form of the journal to which they will be submitted for publication.

Chapter 6 is a summarization of the entire thesis.

An appendix is included following Chapter 6 and consists of additional tables which aid in the comprehension of the thesis.

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CHAPTER 1

INTRODUCTION

Precise color definition has been a difficult assignment for many years. Meat color definition for U.S.D.A. grading standards is described in subjective terms.

Adjectives used in the U.S.D.A. *Official Standards for Grades of Carcass Beef* (1965) to describe the color of lean include: light grayish red, moderately light red, slightly light red, slightly dark red, moderately dark red, dark red, medium dark red, dark red to light brown, very dark, dark, and medium to dark red. These descriptive terms attempt to describe changes in beef muscle color as animals mature caused by quantity or concentration of myoglobin (Mb), but also recognize abnormal conditions such as dark cutting beef.

Just as U.S.D.A. commodity grades serve a function in the efficient marketing of perishable products; accurate, precise, and repeatable color definitions assist in the communication and most efficient marketing of perishable commodities whose consumer acceptance depends to some degree on color. Also, if fresh beef merchandizing by meat packers changes from side, quarter and primal sales to sub-primal, roast and steak sales all degrading color changes will assume increased significance as the size of cut gets smaller (Ledward, 1970).

It is certainly possible that no single factor affects consumer acceptance of fresh beef in retail markets more than color of lean (Hiner, 1954). Various subjective color descriptive terms have evolved as a result of research and some of the most frequently applied already have been listed. Color of lean has long been a determining factor of beef quality and the best color has been described as bright cherry red which develops from a blue or purplish color

very rapidly within the first 30 min after cutting (MacKintosh and Hall, 1935). However, the best beef lean color when kept in optimum conditions of refrigeration will eventually change to brown (Lawrie, 1966; Pirko and Ayres, 1957).

Many current methods of objective physical color measurement are very complex and do not lend themselves to simple interpretation. Matching the color of meat with a colored paddle and designating the resulting color by the appropriate paddle number is probably one of the more simplified methods of color evaluation. A second system involves spinning a disc with various proportions of standard colors attached while making comparisons with the meat surface. Another system or method of objective color definition involves measurement by color reflectance. Color reflectance can be quantified using a photoelectric spectrophotometer complete with a reflectance attachment and may be defined in terms of percent transmission or in absorbance values which are logarithmic functions of percent transmission ($2-\log_{10}$ %T). Color reflectance also is reported in terms of Munsell hue, chroma and value, tristimulus values or Gardner values.

Little work has been done on the use of reflectance spectrophotometry in following fresh beef color changes. Partially because Mb has such a high affinity for oxygen, it is most difficult to measure fresh meat color with precision using reflectance. Other factors which detract from the refinement of reflectance spectrophotometry as a quantitative instrument for fresh beef color include: the reflective nature of cut beef surface texture, presence of connective and/or adipose tissue such as intramuscular fat, variation in sampling area, and sample thickness. In addition, very little information is available on the effect of carcass grade, marbling, and/or maturity on color stability. This experiment was conducted to: 1. determine the effect of bovine maturity and of two different levels of marbling on display case color

stability of fresh prepackaged beef steaks; 2. study reflectance spectrophotometry as a possible objective tool in following fresh beef color change; and 3. study usefulness of reflectance data in predicting muscle Mb concentration.

CHAPTER 2

REVIEW OF LITERATURE

Importance of acceptable color

Judd and Wyszecki (1963) emphasized that each consumer discerns what to buy on the basis of all available senses; touch, taste, smell, sight, and hearing. Naumann et al.(1957) proposes consumer preference for meat is affected by visual and eating preferences. He further indicates visual and eating preferences are summation of consumer experiences which ultimately yield a single choice. The sense of sight is a significant aspect of visual, while senses of taste, smell, and touch are components of eating preference.

Judd and Wyszecki (1963) report that customers perceive color as belonging to the merchandise or to the package; the consumer is considering merchandise color when using color as a selection factor. Birren (1963) and Francis (1963) divided foodstuff's color into two general problem areas; the first dealt with added approved synthetic colors while the second contends with pigments and other factors actually found in the foodstuffs.

Fresh beef in transparent wrappers for self merchandising sells best if it has the natural fresh bright "bloomy" color reflected by oxymyoglobin (Lawrie, 1966; Ramsbottom et al., 1951).

Judd and Wyszecki (1963) affirmed subjective color terms are unimportant, however, the character and amount of difference between the actual color perceived and the mental standard to which it must conform is essential. Lasley et al. (1955) as reported by Nauman et al. (1957) referred to a survey which found nearly one-third of respondents preferred a bright red color of lean for beef. However, when these same respondents were asked to match their bright red with Munsell meat color paddles, eight different paddles were indicated. Hiner (1954) spoke of color as a conditioning characteristic that

largely determines the acceptability factor of a roast or steak, especially beef. Psychological conditioning by color has occurred when an off-colored dark or grayish piece of meat leaves one with a negative response to its acceptability while at the same time a bright red color that shows "life" will have more desirability. Hiner (1954) further indicated "real" conditioning by color is due to associated quality implications such as time held, temperature of holding room, how handled and others all relating to the wholesomeness of meat. The Wisconsin Experiment Station (1963) has published pork color standards but few if any exist for fresh beef.

Meat Color

The color of meat is primarily due to the chemical state of a pigment called myoglobin (AMI, 1960; Schweigert, 1956). Myoglobin changes mainly to oxymyoglobin (MbO_2) during extraction from fresh meat and subsequent analysis (Snyder, 1965). Prolonged exposure of myoglobin (Mb) and MbO_2 to oxygen (particularly at low oxygen pressures and with surface dehydration occurring) results in formation of undesirable brown metmyoglobin (Lawrie, 1966; Schweigert, 1956).

Lawrie (1966) discussed Mb content and found it to be affected by animal species, breed and/or type of species, sex, age, anatomical location and level of muscular activity. He disclosed values of 0.07% Mb from the longissimus dorsi of a 12-day-old calf and 0.46% from the longissimus dorsi of a 3 year year old steer. AMI (1960) reported the Mb content in veal tissue as 1 to 3 mg/gm and in old beef from 16 to 20 mg/gm.

Pirko and Ayres (1957) packaged beef samples in high gas transmissible 0.0381 mm polyethylene film and noted the formation of typical scarlet red color associated with presence of free oxygen in beef. However, after 3 to 6 days this coloration was lost and replaced by typical brown of "old meat."

They spoke of brownish discolored spots of higher metmyoglobin (Mb+) concentration appearing randomly on the cut surface of meat and spreading during subsequent days of storage. Schweigert (1956) referred to green compounds resulting from irreversible oxidation and/or irradiation of Mb+ as well as colorless compounds due to further irreversible oxidation of the green compounds. Lawrie (1966) mentioned three green pigments, sulphmyoglobin, choleglobin, and verdohaem which result from effects of H_2S and oxygen, H_2O_2 and H_2S and oxygen in excess, respectively.

Ledward (1970) reported pigment concentrations of bovine semitendinosus ranged from 4.6 to 6.9 mg/gm of wet tissue. Birmingham et al. (1966) found an average of 4.10 and 3.39 mg Mb/gm for fresh firm and soft bovine longissimus dorsi, respectively. Craig et al. (1959) reported chemically extracted Mb quantities to range from 1.62 to 6.30 mg/gm on a fresh basis for bovine longissimus dorsi. Romans et al. (1965) reported Mb mg/gm of beef rib steaks on a fresh basis in A, B, C, and D maturity levels at 2.72 $\pm$ .12, 3.50 $\pm$ .12, 3.69 $\pm$ .12, and 3.89 $\pm$ .14 respectively. Schweigert (1954) extracted Mb from beef rib and round cuts and noted concentrations between 2.26 and 5.41 mg/gm. Fleming et al. (1960) using Poel's (1949) method of Mb quantitation reported Mb mg/gm fresh basis from 2.33 to 4.11 in beef shank, rib eye, sirloin tip, and heart (mean values: shank 3.22, rib eye 3.51, sirloin tip 2.97, and heart 2.40).

Snyder (1965) reported percent reflectance (component of reflectance ratios used to measure amounts of various forms of Mb present at meat surface during oxidative discoloration) will depend upon the concentration and oxidation state of pigment at the surface of meat.

Percent reflectance at meat surfaces, when incorporated into reflectance ratios similar to those of Dean and Ball (1960) as reported by Snyder (1965),

can indicate amounts of various Mb forms. Ratios such as these are helpful in understanding meat surface discoloration due to oxidation of Mb (Snyder, 1965). Snyder (1965) further pointed out percent reflectance at a meat surface is affected by amount of intramuscular fat, and the amount of surface moisture. Bate-Smith (1948) reported muscles with similar pigment content can vary in color with some appearing lighter in color. This is at least partially due to increased light scattering resulting from meat fibrils shrinking apart as pH is lowered. Briskey et al. (1966) showed pale, soft, exudative porcine muscle had lower pH values and was undergoing the onset phase of rigor mortis prior to one hour postmortem.

Tuma et al. (1962) reported postmortem aging for 14 days produced a brighter more intense red color in beef longissimus dorsi as evidenced by increases in Munsell value and chroma with correspondingly lower hue. Tuma et al. (1962) also concluded that marbling may not necessarily cause more light to be reflected, as some have surmised, since they noticed slight increases in Munsell hue and chroma associated with slight decreases in value as marbling increased. However, all Munsell notation changes in their study were not significantly affected by marbling.

Chemistry of fresh meat pigments

In sterile normal lean bovine tissue any color present is due mainly to the amount and chemical state of the heme proteins Mb and hemoglobin (Hb). Myoglobin is a complex protein. A single Mb conjugated protein molecule is composed of a globin protein portion and a non-protein portion called heme. The globin protein has a molecular weight of 16,000-17,000 (AMI, 1960; Schweigert, 1954). Myoglobin's function in the live animal is to accept oxygen from Hb of blood for use in oxidative energy yielding reactions in the cell (Schweigert, 1954).

Schweigert (1954) further reports that the heme portion is composed of two parts: an iron atom and a large planar ring called porphyrin. The iron atom has six bond orbitals in which the iron is sharing two electrons with another atom. Five electron pairs are often shared with nitrogen atoms and one with oxygen. The bond type formed with the oxygen bond orbital and other chemicals is the most important factor in determining the final color of the complex. The oxidation state of the iron and the physical state of the globin are important in determining the final color (Lawrie, 1966; AMI, 1960). Covalent bond complexes plus other factors are responsible for the bright red color of fresh postmortem meats. Oxymyoglobin is an example of ferrous covalent complex of Mb and oxygen. In fresh postmortem meat MbO_2 represents the bright red color desired by purchasers according to Lawrie (1966). In the presence of oxygen, Mb is reversibly converted to either of two different pigments; MbO_2 and Mb+, the oxygenated and oxidized pigment forms respectively. Iron remains in the Fe++ state while part of Mb and MbO_2; it is changed to the Fe+++ state in Mb+ (Lawrie, 1966). Oxidation of enzymatic substrate, particularly glucose, provides a continual supply of reducing coenzymes capable of reducing Mb+ or MbO_2 back to Mb (AMI, 1960). Oxymyoglobin will persist on the surface of fresh postmortem meat as long as a plentiful supply of oxygen and reducing substances exists. Also, as long as the muscle supply of oxidizable substrates lasts, interior heme pigments will be retained in a dark purple reduced state.

Color brightening (Mb oxygenation) and subsequent deterioration (MbO_2 further oxidized), basically a chemical reaction, can be influenced by many factors. Since typically normal color changes are basically due to the combination of Mb and oxygen, the kinds and partial pressures of gases in the atmosphere surrounding the lean meat influence color of postmortem beef muscle.

Absence of oxygen, and the presence of reducing conditions, keeps Mb in the reduced state, purplish red in color (Schweigert, 1954). Pirko and Ayres (1957) found beef round muscle pigments in a reduced state through a 14 day storage period when packaged in films having low rates of gas transmission. Hansen and Sereika (1969) obtained Mb representative reflectance spectra by vacuum packaging bovine gluteus medius in a Cryovac Saran S pouch, holding 1 hr, and allowing reducing system of meat to operate.

Ledward (1970) reported virtually constant bovine semitendinosus Mb+ concentration between 5 and 14 days storage at 0 and 7 C, at gaseous atmospheres of air or air plus 12% carbon dioxide, and 5.74 to 5.8 pH levels. He reported equilibrium concentration of Mb+ at the surface to be independent of surface pH. Ledward (1970) revealed formation of maximal quantities of Mb+ in bovine semitendinosus at partial oxygen pressures of 7.5 ± 3 and 6.0 ± 3 mm Hg at 7 C and 0 C respectively when comparing oxygen pressures ranging from zero to 160 mm Hg.

Many chemicals alter the color of beef lean. Snyder (1965), Stewart et al. (1965) and Hansen and Sereika (1969) used a highly oxidizing chemical, $K_3Fe(CN)_6$ to cause a brown color on the muscle surface. At the same time nitrates, nitrites, and ascorbates used in curing meat and meat products aid in the development and stabilization of a cured red color (nitrosomyoglobin), as noted by Lawrie (1966), Sair (1962), and Schweigert (1956). Stewart et al. (1965) converted meat pigment to Mb by adding sodium hydrosulfite, while Dean and Ball (1960) used sodium dithionate ($Na_2S_2O_4$).

Lawrie (1966) revealed three chemical forms of Mb (sulphmyoglobin, choleglobin, and verdohaem) that generally were the consequence of deteriorating effects of hydrogen sulfide, hydrogen peroxide and oxygen on Mb or MbO_2 (choleglobin can result from action of ascorbate or other reducing agents

on MbO_2).

Light energy affects meat color. Marriot et al. (1967) found fresh beef muscle retains desirable color longer when stored in complete darkness as compared to lighted display. Hansen and Sereika (1969) indicated drastic shortening of display case life with frozen beef illuminated to 4.3×10^3 lumens/m^2 as compared to illuminations of 2.15×10^3 lumens/m^2. Ramsbottom et al. (1951) showed bovine longissimus dorsi steaks were not discolored after 36 hr exposure to 6.45×10^2 lumens/m^2 fluorescent light but their color did change after 36 hr exposure at a distance of 0.61 m from a 30 watt germicidal lamp emitting ultra-violet rays. Schweigert (1956) pointed out that irradiation and light were catalytic to the irreversible oxidation reactions yielding so called "green compounds."

Methods of measuring meat color changes

In an effort to objectively evaluate the chemical forms responsible for various colors of lean muscle without laborious chemical extraction and eventual sample destruction, many reflectance spectrophotometric measurements have been made on samples containing Mb and/or some of its forms (Dean and Ball, 1960; Ledward, 1970; Naughton et al., 1958; Pirko and Ayres, 1957; Snyder and Armstrong, 1967; Snyder, 1965; Stewart et al., 1965). A great number of the reflectance studies have been concerned with quantitatively determining Mb, MbO_2 and Mb+.

As early as 1957 Naughton et al. tested adherence of spectral reflectance to Beer's law by using 2-14 mg of copper sulfate solutions added to crystaline alumina diluent. They found such adherence to Beer's law with varying concentrations studied at 620 nm. Also, they measured the absorbancy of the heme protein system of tuna fish flesh and whole human blood. Between 380 and 660 nm, they found wavelengths of absorption maxima in reflectance to correspond

exactly to those found in transmission. Naughton et al. (1957) revealed Soret absorbancy values from 2.0 to 2.2 between 400 and 420 nm for tuna flesh with mixed MbO_2 and Mb+ and with Mb+ only.

Naughton et al. (1958) obtained absorption from reflectance curves on fresh and fresh frozen thawed tuna flesh pigments between 400 and 700 nm (visible range). He expected a green sample to show greater absorption of light in the red end of the spectrum than in the green, when compared to normal flesh. He used ratio at 540 (green) to that at 640 (red) nm on cooked green and normal flesh and expected it to be greatest for normal tuna and least for green meat. He calculated ratio values ranging from 1.4 to 2.0. The 410 to 415 nm absorbancies (Soret peak) ranged from 0.6 to 1.2. When plotting A540/A640 nm peak ratio vs. absorbancy at Soret peak (410-415 nm), the most assumed characteristic peak for heme pigments indicating the absolute pigment content of the cooked meat, he found normal samples generally occupied that portion of the figure indicative of higher A540/A640 ratios and lower pigment content (white albacore low pigment tuna was considered just as normal as fleshy pink and both were associated with high A540/A640 ratios).

Pirko and Ayres (1957) packaged 1.27 cm thick U.S.D.A. Choice bovine biceps femoris and semimembranosus muscles in various meat films, stored them and measured their reflectance between 30 min and 14 days post cutting. They used 2% KCl to extract pigment from samples adjacent to those used for reflectance. A grayish-white highly reflective substance remained and was used to discover if maximal reflectance values were due to pigments or pigment carriers. Reflectance values in the region 500-580 nm were between 58 and 61% for the pigment extracted gray white substance, while fresh beef values were much lower at 2 to 4%. As a result, they postulated that lower pigment content resulted in higher reflectance at all wavelengths studied.

Mean reflectance percent at 635 nm of six beef samples packaged in high gas transmissible 0.0381 mm polyethylene decreased after initial cutting. Minimal reflectance at 635 nm (approximately 13 to 14%) on these same samples occurred at approximately the sixth day of storage. Maximal reflectance at 580 nm (approximately 8 to 9%) occurred on the sixth day. Spectral reflectance values at 555, 580, and 635 nm showed very little range, approximately 1 to 5%, between the 8th and 14th day.

Dean and Ball (1960) used K/S values as suggested by Judd (1952) on 5.72 cm diameter circular samples of U.S.D.A. Choice, Kosher slaughtered unpackaged beef held at 1 C with atmospheric conditions not specified. K is absorption coefficient or fraction of incident energy lost by absorption per unit of concentration per unit thickness of material and S is scattering coefficient or fraction of incident light lost due to scattering per unit thickness of material. This procedure tested the validity of a reflectance ratio method modification made on the absorbancy ratio method of Broumand et al. (1958). Dean and Ball (1960) obtained top surface reflectance measurements at 473, 507, 573, and 597 nm completed approximately 38 sec after muscle exposure to air. Reduced Mb measured by the reflectance ratio method represented over 50% of the total of three Mb forms on five of the six raw unpackaged samples measured approximately 38 sec after cutting (sixth was 47%). The percentage of Mb+ on the same samples varied from zero to under 10%. However, for these same surfaces, the absorbancy ratio method indicated that reduced Mb represented only about 20%, while Mb+ represented 20% and MbO_2 was over 50% of total pigment. Absorbancy determinations were made an average of 6 min after exposing the freshly cut beef surface and required an average of 10½ min to complete. Dean and Ball (1960), after a second group of absorbancy ratio measures were completed immediately after exposing the fresh meat surface, concluded that

the reflectance method was giving truer values for composition of the meat sample surface but each method was valid for special circumstances.

Snyder (1965) recorded reflectance spectra of fresh U.S.D.A. Choice beef round steak purchased from a local market. He examined spectra in the wavelength range of 400 to 700 nm using the absorbancy scale. In an effort to find reproducible isobestic points (the wavelength at which absorbancy indices of two or more pigments are identical), Snyder (1965) encountered data scatter and subsequently adjusted all spectral curves to a common reflectance (in absorbance units) of R_A = 1.0 at 525 nm. He justified this adjustment stating that it provided information on relative percentage values of Mb derivatives and not absolute amounts of the pigment. Snyder (1965) reported isobestic points for Mb+ and MbO_2 at approximately 474 nm, and for Mb and MbO_2 at approximately 571 nm. Further, he stored beef samples up to 8 days at 0 C and followed color deterioration. He indicated very slow early changes for R_A at 571 nm in comparison with Gardner a values, however increased change occurred as storage time increased. Snyder (1965) attributed this slow R_A change at 571 nm to fresh beef color changes beneath the surface which were not measured by reflectance due to sample illumination with very low intensity monochromatic light. To make reflectance values more sensitive to color changes beneath the surface of fresh beef, he suggested wavelengths in the "red region (particularly 630 nm)" be used to detect changes from MbO_2 to Mb+. He also reported much larger changes of stored fresh beef reflectances at 631 nm in relation to those at 571 nm; probably due to the greater penetrating power (less scattering) of longer wavelengths. Also, Snyder (1965) noted very little R_A change at 474 nm over the 8 day period.

Snyder and Armstrong (1967) found both the adjustment of R_A = 1.0 at 525 nm followed by use of K/S values compared to use of only the K/S technique

of Dean and Ball (1960) to be equally effective in decreasing results of unwanted light scatter in reflectance data. However, they concurred the K/S ratios were preferred to arbitrary adjustment of reflectance data prior to obtaining a K/S value. When R_A and K/S values of varying concentrations of MbO_2 and Mb in dried milk model systems were plotted, the K/S values demonstrated much more linearity as compared to the R_A values.

Stewart et al. (1965) used locally purchased trimmed and twice ground beef (rib, chuck, and round) and pork (loin). To study the spectral characteristics of pigment-free meat, some samples were bleached. The bleached meat spectra showed no peaks in their absorption spectra, but gradual increase in absorption from longer to shorter wavelengths in the region of 700 to 450 nm was exhibited. These same spectra would demonstrate a gradual decrease in reflectance percent from longer to shorter wavelengths in the region of 700 to 450 nm. They also mentioned bleached cooked meat spectra to be of the same general shape as raw but always with "higher absorbancies" than corresponding raw samples. Further, they agreed on an isobestic point for all three Mb pigment forms at 525 nm and an isobestic point for Mb and MbO_2 at 572 or 573 nm. Stewart et al. (1965) reported a range of K/S ratio at 572 nm vs K/S at 525 nm for Mb+ to be 0.51 to 0.65 and for Mb between 1.13 and 1.61. Stewart et al. (1965) adjusted pH in a ground beef sample from 6.5 to 5.1 and noticed a 525 nm K/S value decline from 4.42 to 2.37.

Ledward (1970) studied "aseptically" cut beef semitendinosus which had been removed from the carcass directly after slaughter, "flamed" and aged for 2 days under several conditions. Initial experiments were concerned with various percentages of Mb+ and a combination of various pH levels with a range of Mb+ percentages. In other experiments, he used a controlled storage environment of a continuous flow of air or nitrogen (both containing 10% CO_2)

maintained at a relative humidity of 99.3% and temperature of 0 to 7 C. Prior to recording reflectance spectra from 380 to 770 nm, Ledward (1970) exposed all samples to air 2 to 3 hr. He reduced standard deviation by using spectral adjustment R_A = 1.0 at 525 nm prior to calculating K/S values, as compared to standard K/S values. He reported pigment concentrations ranging from 4.6 to 6.9 mg/gm of wet tissue. Due to error arising from a characteristic spectrum of intramuscular fat, Ledward (1970) suggested muscles with different fat contents cannot be compared by spectral reflectance.

Effect of maturity, marbling, quality on meat color

Romans et al. (1965) found significantly ($P < .05$) higher Munsell hue means for moderate marbled steaks when compared to slight marbling levels. Differences in Munsell chroma as affected by marbling were not significant. Even though carcass maturity levels studied had no effect on Munsell hue and chroma, the A maturity level Munsell value mean at 4.05 $\pm$ .05 was significantly ($P < .01$) higher as compared to the other maturity levels (B, C, and D). Tuma et al. (1962) found the three Munsell dimensions of color in general, indicated that longissimus dorsi became darker red as animal age advanced. They reported significant decreases in all three Munsell notations of hue, value, and chroma ($P < .005$, .01, and .05 respectively) as animal age increased from 18 to 90 mo. Romans et al. (1965) reported no significant difference between the two levels of marbling with respect to quantities of total pigment, Mb, and/or Hb. Myoglobin, on a fresh tissue basis, of the A maturity group was significantly ($P < .01$) lower than other maturity groups examined. Although not significant, quantitative Mb values tended to increase with increasing maturity for the other maturity groups. Of the color measurements used, only Munsell value was correlated at -.45 ($P < .01$) with Mb concentration. Also, Romans et al. (1965) reported a significant ($P < .05$) positive correlation ($r = 0.25$)

of Mb quantity to Hb quantity, both on fresh basis.

Birmingham et al. (1966) reported on the stability of 1.90 cm thick bovine longissimus dorsi cut from U.S.D.A. Choice and Good short loins with "firm" and "soft" muscle. The fresh steaks were wrapped in 300 MSAT 80 cellophane and stored between temperatures of 0 and 12 C with 645 to 700 lumens/m^2 of continuous light exposure. Muscle firmness was evaluated both objectively using a "Precision" universal penetrometer and subjectively. Color of samples was appraised by a five member panel on an eight point hedonic scale for over-all desirability of color. Myoglobin was extracted according to a procedure reported by Ginger et al. (1954). They indicated beef firmness was not significantly associated with color degradation. They also found firm and intermediate fresh pork retained desirable color longer as compared to soft pork.

Hunt (1970) found no significant difference in color subjective values or reflectance spectra between 400 and 700 nm for frozen lamb loin chops of 3 marbling levels, namely moderate or slightly abundant, slight or small, and practically devoid or devoid marbling.

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CHAPTER 3

REFLECTANCE SPECTROPHOTOMETRIC CHANGES OF FRESH PREPACKAGED BOVINE LONGISSIMUS DORSI MUSCLE DURING "BLOOM" AND SUBSEQUENT DISCOLORATION

INTRODUCTION

A freshly cut surface of bovine muscle when exposed to air changes color from the relatively darker purplish red of reduced myoglobin (Mb) to the relatively bright red of oxygenated myoglobin (MbO_2) and eventually to the predominantly brown metmyoglobin (Mb+).

Mackintosh and Hall (1935) and others have reported oxygenation or "blooming" of meat occurs very rapidly within the first 30 min after cutting and is accelerated by reducing the temperature. Dean and Ball (1960), using the absorbancy ratio method of Broumand et al. (1958) on exposed surfaces of fresh unpackaged U.S.D.A. Choice beef samples between 6 and 10½ min post cutting, found over 50% MbO_2 present. On the same samples, reflectance ratio, measured prior to the absorbancy ratio and 38 sec after cutting, showed over 50% Mb. These samples were from carcasses chilled at 2 C for 7 to 10 days prior to sampling. Pirko and Ayres (1957) showed a rapid decrease in percent reflectance at 635 namometer (nm) of U.S.D.A. Choice bovine biceps femoris and/or semimembranosus packaged in 0.0381 mm polyethylene between initial cutting and approximately 4 hr post cutting. Also, they described how this packaging film permitted the formation of "the typical scarlet red color associated with the presence of free oxygen in beef" which after 3 to 6 days was lost and replaced by the "brown color typical of old meat." Snyder (1965) presented spectra from adjacent slices of beef round treated to form predominantly Mb, MbO_2, or Mb+ at the surface and showed a gradual general decrease in R_A (reflectance measured on absorbancy scale) between 400 and 700 nm.

Also, Snyder (1965), studying 20 fresh beef samples as they deteriorated in color while stored in air between 0 and 2 C, noticed very little R_A change at 474 nm over an 8 day period whereas R_A at 571 nm decreased very slowly at first but recorded faster decreases as storage time increased. Snyder (1965) showed immediate Gardner a value (red-green scale) changes after storage began. Ledward (1970) revealed beef semitendinosus stored under carefully controlled conditions of temperature, relative humidity, and air microbial contamination, attained maximum percent Mb+ on about the 5th day of storage.

A primary consideration of this study was to further appraise the use of reflectance spectrophotometry as a possible objective tool in following fresh beef color changes.

EXPERIMENTAL PROCEDURE

The longissimus dorsi muscle from the left wholesale rib cut of 60 steer carcasses (241 to 438 kg) was studied. Carcasses of unknown history were selected in commercial packing plants at 24 hr postmortem to represent both small and moderate marbling levels within each of three U.S.D.A. physiological age groups; A-A, A+B-, and B B+ (referred to as young, intermediate, and approaching maximum maturity, respectively). Degree of maturity was determined by the amount of bone ossification and color of lean as defined by the Federal Grading Service (U.S.D.A., 1965). Color of lean was used only as a "sample elimination factor" if it did not conform to the color typical for a maturity group. "Dark cutting" carcasses were not included in this study. Ten carcasses were selected per experimental cell.

The wholesale ribs were transported to Kansas State University meat laboratory and cut at 10 days postmortem. A rib steak approximately 5.08 cm thick was removed over the 8th thoracic vertebra. The longissimus dorsi

muscle was separated; cut into two 2.54 cm thick portions immediately before the first objective color reading. Time elapsed between cutting the longissimus into two pieces and beginning the first objective color reading never exceeded 30 sec.

The freshly cut (anterior) surface of the posterior half of longissimus dorsi muscle sample was used for subjective and objective color measurements. This steak was wrapped in oxygen permeable fresh meat cellophane with an opaque fiber board backing and sealed. The packaging film was approximately 0.028 mm thick, with about 0.006 mm low density polyethylene on a base sheet of cellophane. Fresh steaks were stored in approximately 860.8 lumens/m^2 of incandescent light at about 6 C.

Both subjective (Appendix tables 1 and 2) and objective color measures were made at 26 time intervals; namely, immediately after cutting; 5, 10, 15, and 20 min; and 1, 2, 3, 10, 24, 48, 72, 96, 128, 136, 149, 154, 158, 163, 173, 178, 182, 187, 192, 216, and 240 hr post cutting.

Subjective color values were estimated in about 484.2 lumens/m^2 of cool white fluorescent light and on a white cotton background, using an eleven point scale (figure 2). Plate I illustrates typical purplish red Mb (visual value 2.0), typical bright red MbO_2 (visual value 5.0), and typical flat or satin brown Mb+ (visual value 9.0) as defined for use in this study.

Color reflectance was recorded in the visible light range of 400 to 700 nm on a Bausch and Lomb 600 reflectance spectrophotometer at a scan speed of 250 nm/min using a MbO block wrapped in fresh meat cellophane for 100% reflectance. Care was taken to scan the same portion of sample surfaces each time and to exclude large fat or connective tissue areas from the sampled area. Duplicate reflectance scans were not recorded as color changes during the early time intervals were too rapid.

EXPLANATION OF PLATE I

Typical longissimus dorsi subjective colors; Top - purplish red myoglobin (Mb, visual value 2.0), Middle - bright red oxymyoglobin (MbO_2, visual value 5.0), and Bottom - brown met-myoglobin (Mb+, visual value 9.0).

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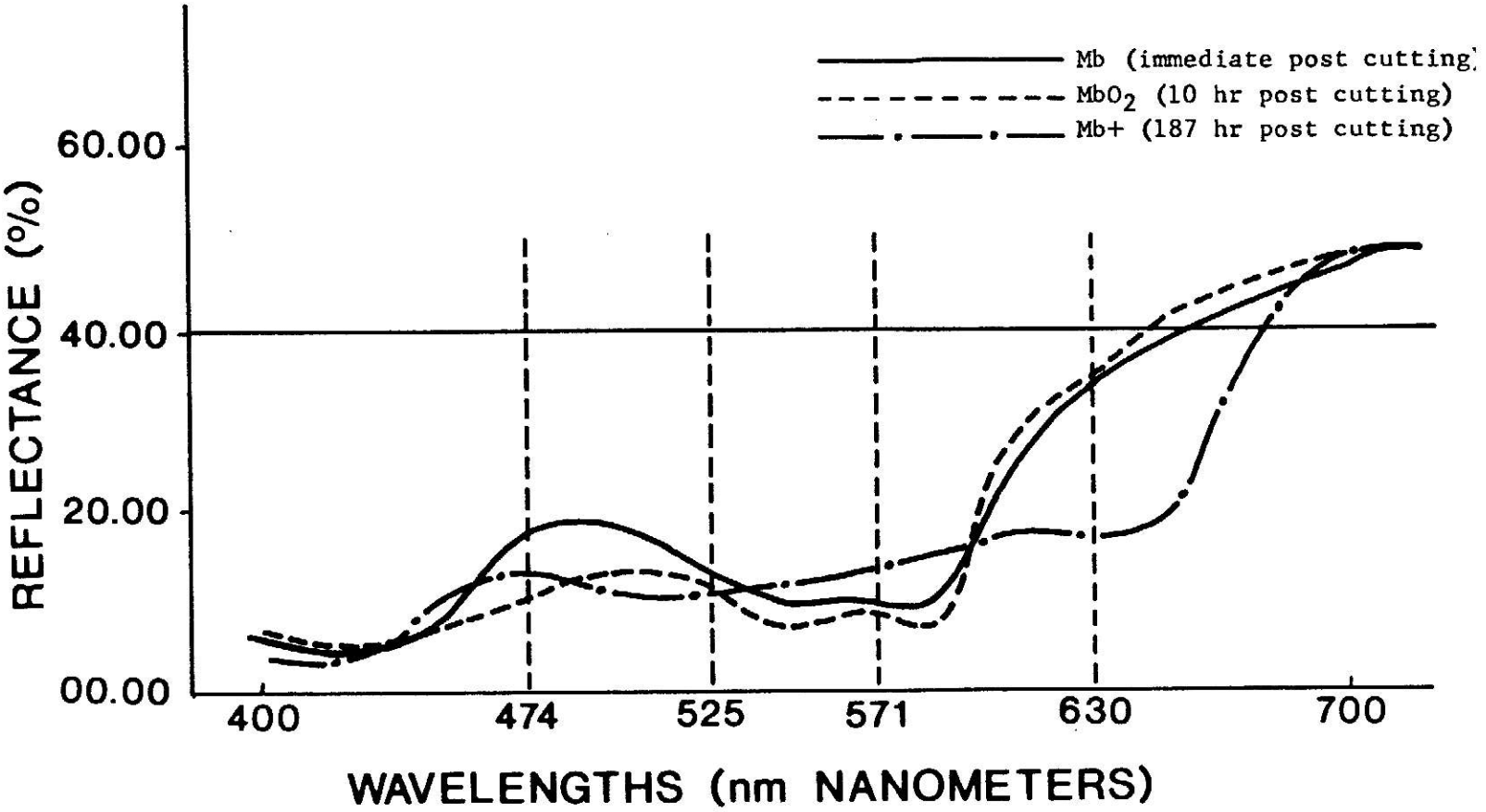

Fig. 1. Typical reflectance scans of fresh bovine longissimus dorsi.

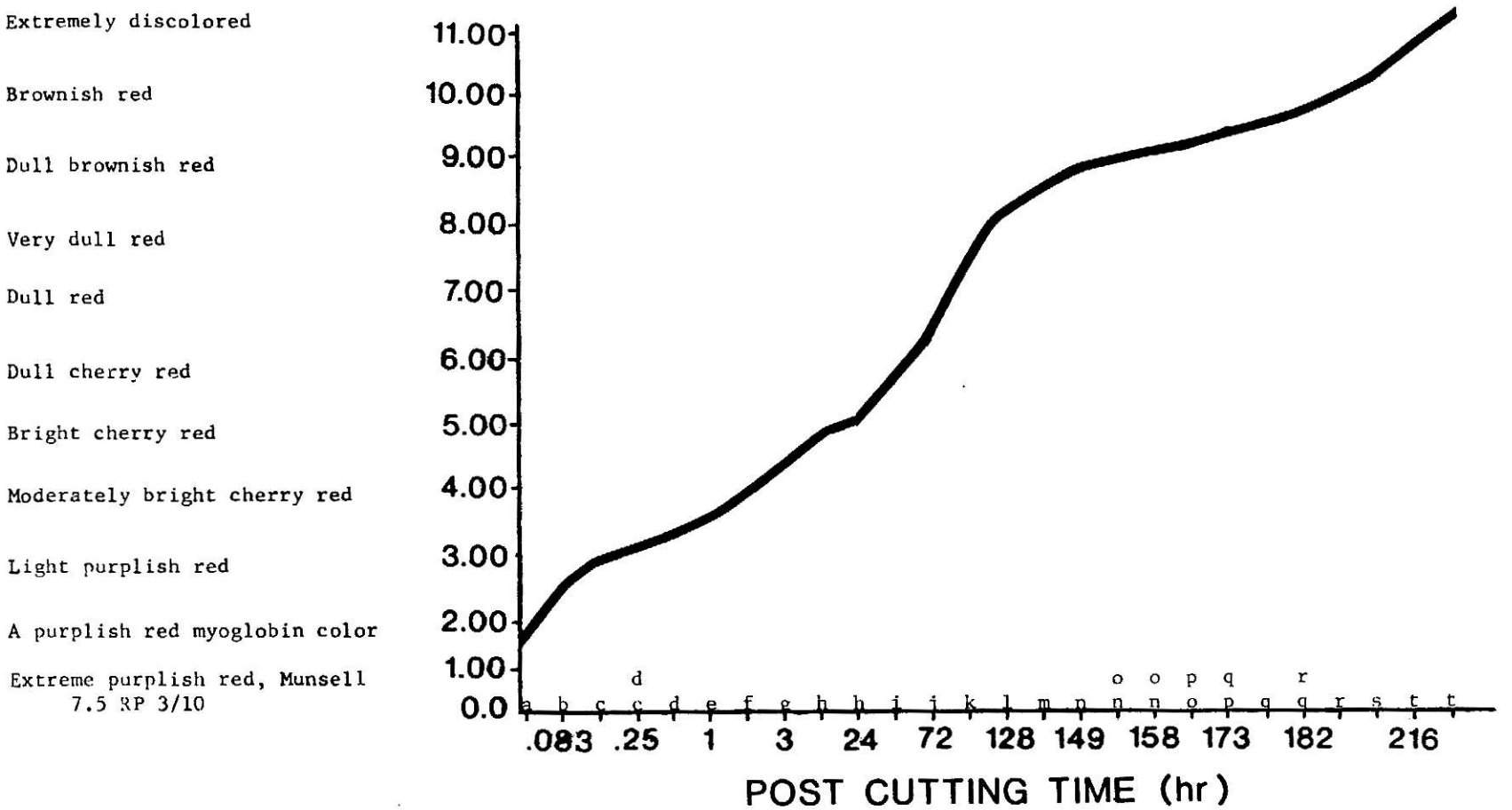

Fig. 2. Subjective visual score means of fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-t Time means with same letters not significantly different ($P < .05$)

Analysis of variance and least significant difference (LSD) procedures were used to detect differences between time period means for percent reflectance at each of nine light wavelengths (474, 525, 538, 568, 571, 600, 610, 620, and 630 nm), for two reflectance ratios (R474/R525 nm, R571/R525 nm), and for subjective visual values.

RESULTS AND DISCUSSION

The steaks in our experiment changed visual color as expected. A purplish red color was evident immediately post cutting with an overall visual mean (60 samples) of 1.75 (figure 2). Maximum bright red color developed somewhere between 3 and 24 hr post cutting with a visual mean of 4.3 at 3 hr and 5.0 at 24 hr. Brown Mb+ color appeared between 96 and 163 hr under these conditions with a pattern similar to those mentioned by Pirko and Ayres (1957).

Figures 3 through 8 illustrate the mean percent reflectance for each reading time for all 60 steaks at the nine wavelengths and two wavelength ratios studied. Each point is equally spaced although a wide divergence occurred between adjacent reading times.

Mean reflectance percent at 474 nm (figure 3) showed a marked and significant ($P < .05$) decrease from 0 time to 5 min post cutting but with no further significant difference through 48 hr of display. A gradual increase in reflectance percent at 474 nm occurs from 96 through 240 hr. Both patterns seem to conflict with Snyder's (1965) data reporting very little R_A change at 474 nm during 8 days of storage. Steaks with both Mb predominating (0 time) or Mb+ (later time periods) possess greater light reflectance at 474 nm than those with primarily MbO_2 (3-24 hr time) predominating. Minimal mean reflectance of 10.28% occurred at 15 min post steak cutting and the reflectance values at 5, 10, and 20 min, and at 1, 2, 3, 10, 24, and 48 hr were not statistically

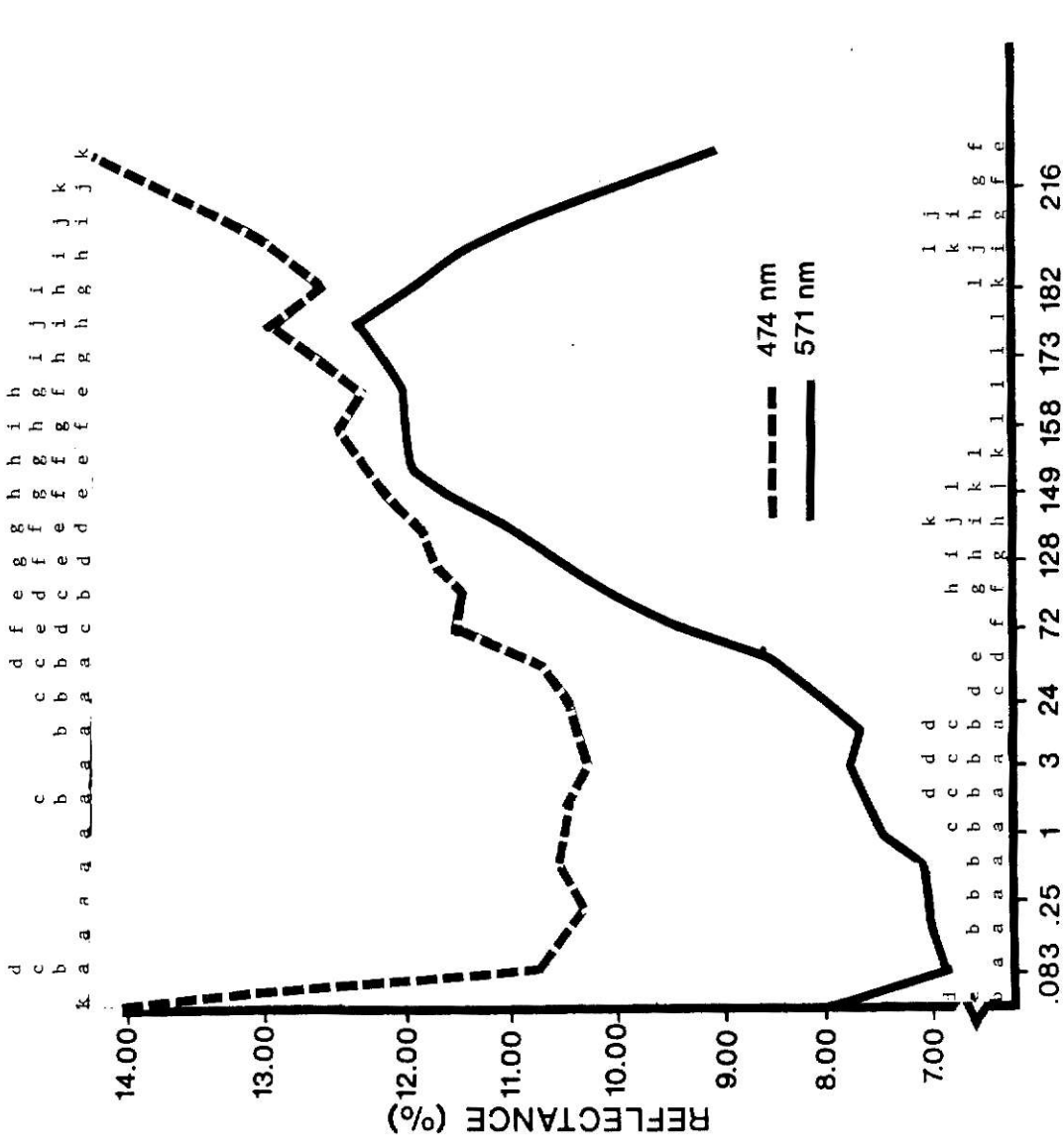

Fig. 3. Percent reflectance means at 474 and 571 nm for fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-l Time means with same letter not significantly different ($P < .05$)

different from this minimum.

Reflectance percentages at 525 nm (figure 4) exhibited a significant ($P < .05$) decrease from 0 time to 5 min but the change is less than at 474 nm. The smallest range in mean percent reflectance of all wavelengths studied was noted at 525 nm (1.59% total range). Between 24 and 240 hr, means are not significantly different. These data tend to agree with the isobestic nature of this wavelength for the three myoglobin forms reported by Snyder (1965), and Stewart et al. (1965).

The mean reflectance percent pattern at 538 nm (figure 4) also presented a significant ($P < .05$) decrease from 0 time to 5 min post cutting, then did not increase significantly through 24 hr of display. Between 48 and 72 hr periods, a significant ($P < .05$) increase in reflectance occurs. A gradual increase in 538 nm reflectance percent was observed between 10 and 192 hr after exposure to oxygen. Maximum reflectance percent at 538 nm occurred at 178 hr, but percentages between 158 and 192 hr are not significantly different from that value.

Reflectance maxima at 568 nm (figure 5) occurred between 149 and 182 hr with the highest value of 12.52% at 178 hr. Minimum reflectance of 7.02% appeared at 5 min post steak cutting, but those at 5, 10, 15 and 20 min, 1, 2, and 10 hr were not statistically different. As at the other wavelengths discussed, reflectance at 568 nm exhibited a significant ($P < .05$) decrease between a value of 7.99% at 0 time and 7.02% at 5 min post cutting.

The mean reflectance percent pattern at 571 nm is shown in figure 3. Again a significant ($P < .05$) decrease from 0 time to 5 min is noted. From 48 hr to 72 hr a significant ($P < .05$) increase from 8.4 to 9.4 was noted. This latter increase occurred when mean visual score changed from maximum bloom to initiation of flat brown visual score. Otherwise, reflectance

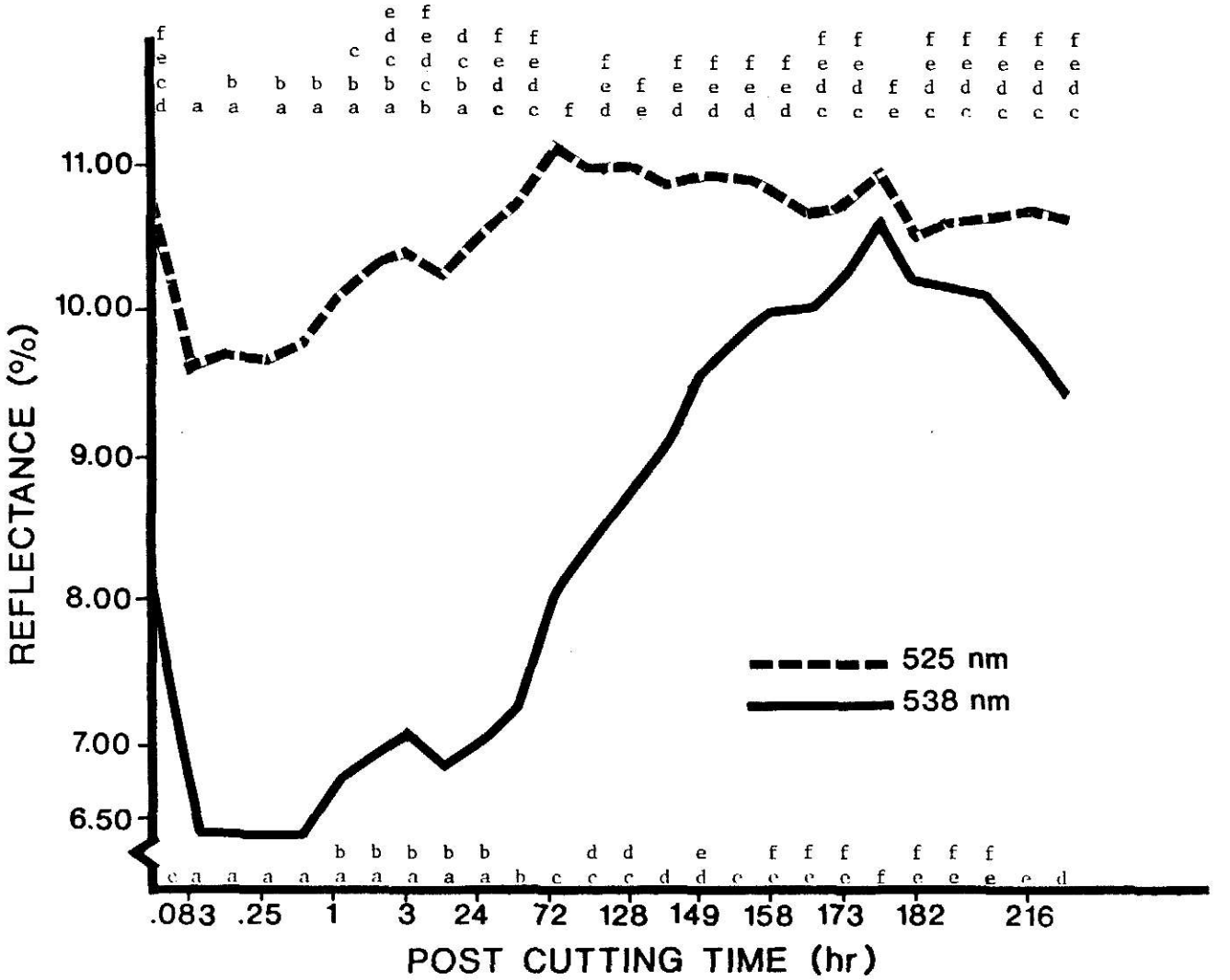

Fig. 4. Percent reflectance means at 525 and 538 nm for fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-f Time means with same letter not significantly different ($P < .05$)

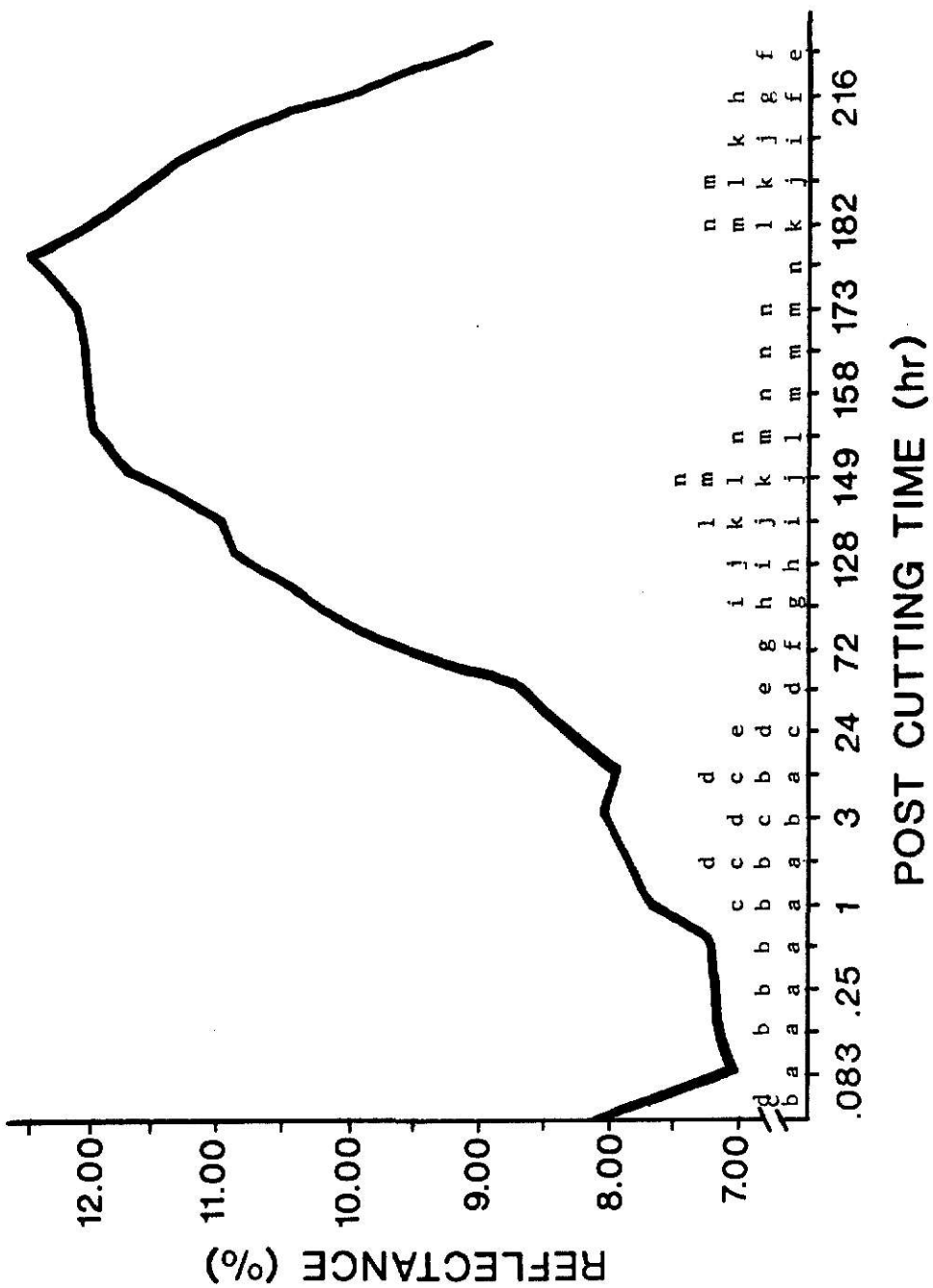

Fig. 5. Percent reflectance means at 568 nm for fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-n Time means with same letter not significantly different ($P < .05$)

percentages at this wavelength appear to be insensitive in relation to visual color changes. The data presented for 571 nm seem to contradict Snyder's (1965) observations at this wavelength made on fresh beef stored for 8 days.

Reflectance percentages at the longer wavelengths (600, 610, 620, and 630 nm) did not show significant decreases between 0 and 5 min as did other shorter wavelengths, although a trend toward increased reflectance was shown. At 600 nm (figure 6) the greatest mean reflectance occurred at 48 hr just after visual values indicated maximum appearance of bloom. This reflectance value of 23.46% was not significantly different from that at 10, 24, or 72 hr. At 610 and 620 nm, shown in figure 7, maximum mean reflectances of 29.83% and 31.96% respectively occurred at 24 hr; the same period a mean visual value of 5.0 (bright red) occurred. Maximum reflectance at 630 nm was 34.38% occurring at 10 hr post cutting, however, reflectance percentages at 1, 2, 3, and 24 hr were not statistically different. Muscle in a predominantly Mb+ state had lower light reflectance at these wavelengths than those with Mb or MbO_2 predominating.

Reflectance ratio at R474/R525 nm (figure 8) displayed significant ($P < .05$) decreases in value between 0 time and 10 min. A gradual increase in value between 24 and 240 hr time periods corresponded to visual color deterioration during the same period. Ratio R474/R525 nm appeared to be more useful in following fresh beef color brightening as compared to ratio of R571/R525 nm also shown in figure 8.

Dean and Ball (1960), using wavelengths of 473, 507, 573, and 597 nm, eluded to some conversion of Mb to MbO_2 immediately after a fresh meat surface is exposed. Data at 474, 525, 538, 571 nm and ratio R474/R525 nm of this research conclusively demonstrated a definite reflectance change occurring at the sample surface between immediately post cutting and 5 min post cutting.

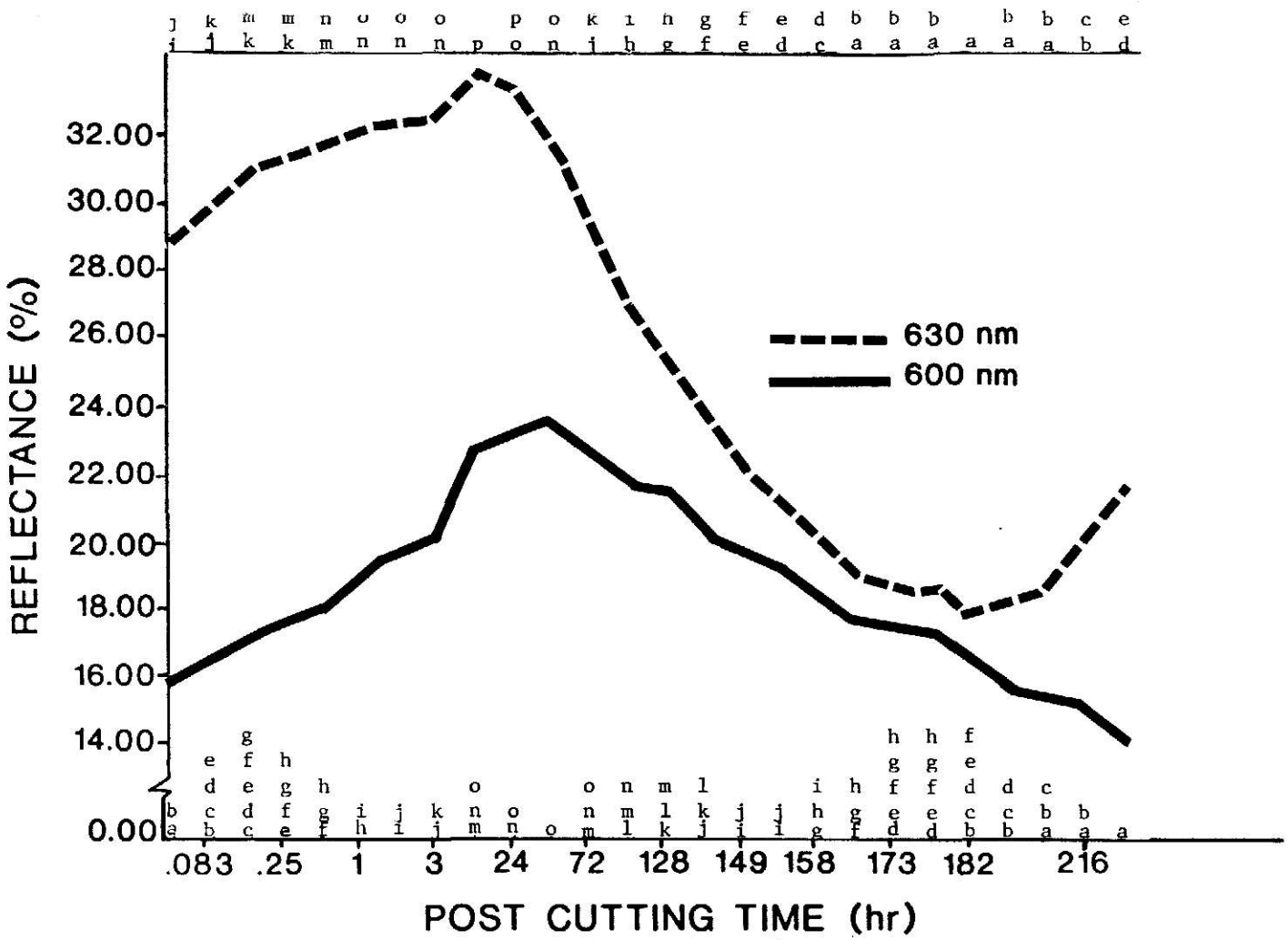

Fig. 6. Percent reflectance means at 600 and 630 nm for fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-p Time means with same letter not significantly different ($P < .05$)

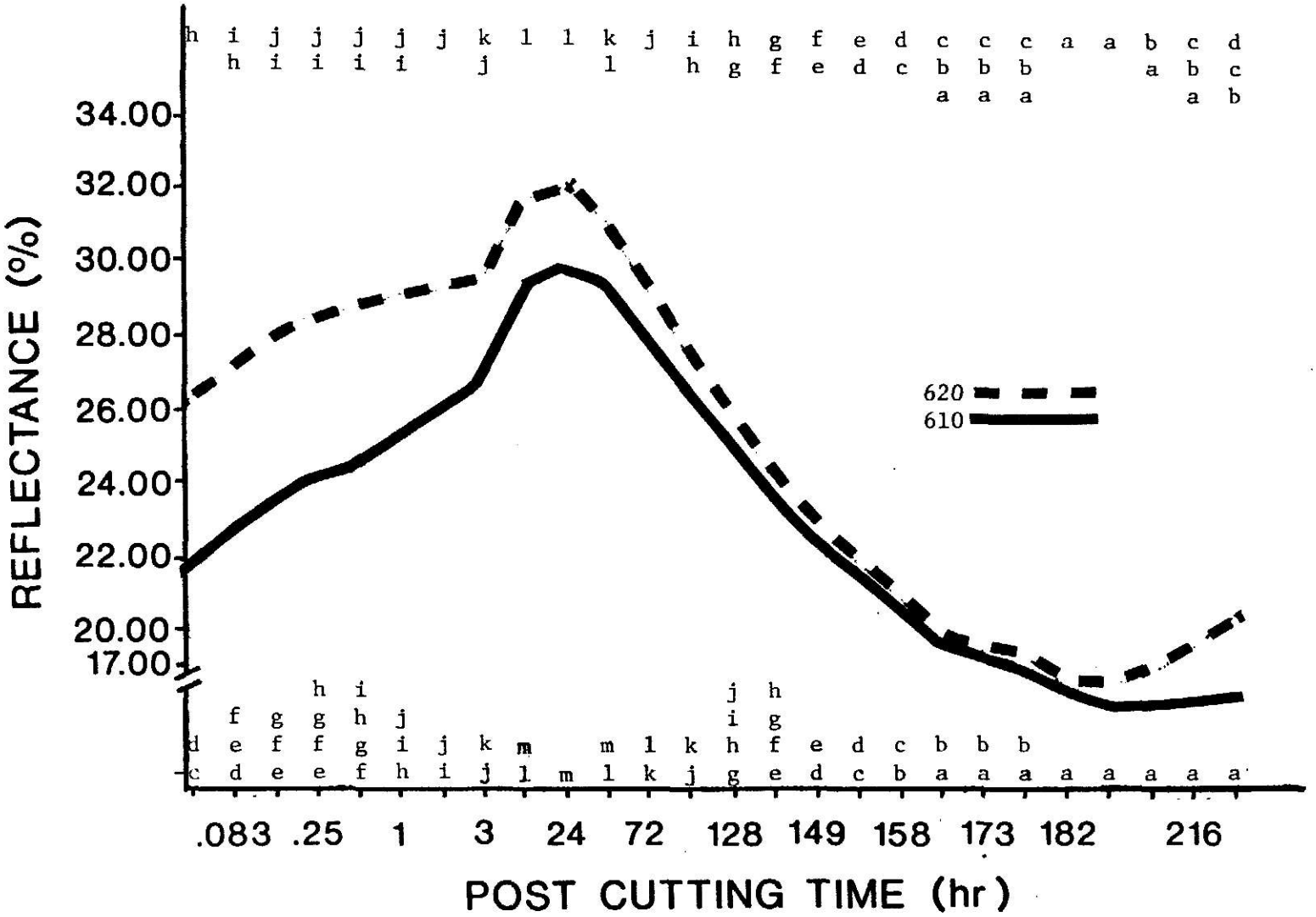

Fig. 7. Percent reflectance means at 610 and 620 nm for fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-m Time means with same letter not significantly different ($P < .05$)

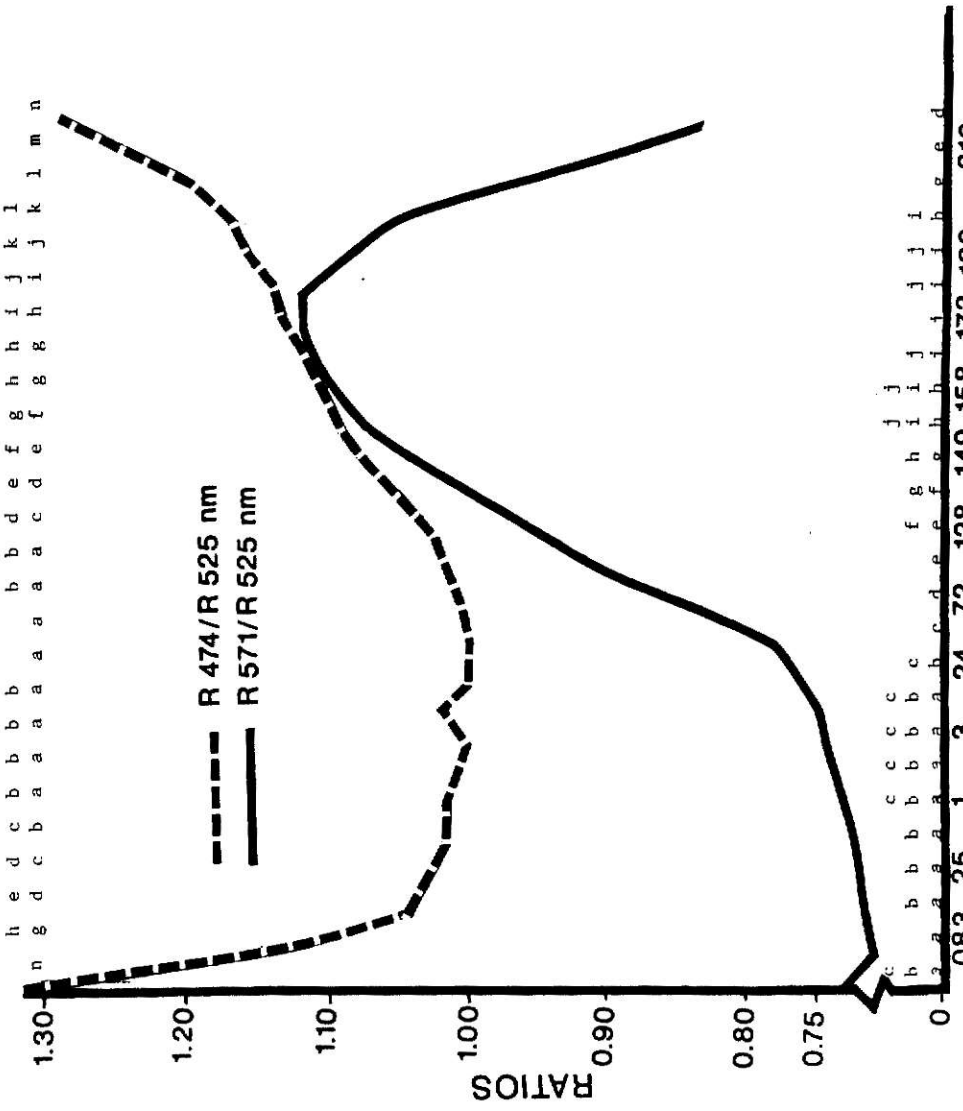

Fig. 8. Ratio means for R474/R525 and R571/R525 nm for fresh prepackaged bovine longissimus dorsi after various post cutting intervals at 6 C, 860.8 lumens/m^2 incandescent lighting.

a-n Time means with same letter not significantly different ($P < .05$).

The post cutting time pattern for percent reflectance at 474, 600, 610, and ratio R474/R525 nm showed possible two slopes indicating a similarity between steaks in predominantly Mb or Mb+ form. Therefore, perhaps these objective measurements did not distinguish between predominantly Mb and Mb+ states of beef longissimus dorsi steaks, although some appear to distinguish between MbO_2 and the other two forms.

SUMMARY

Cellophane wrapped fresh longissimus muscles from the left wholesale rib cuts of 60 steer carcasses representing two marbling levels (small and moderate) and three maturity groups (A-A, A+B-, and B B+) were used to make subjective and objective color evaluations at 26 time periods ranging from 0 time to 240 hr post cutting. Steaks were displayed at 6 C under 860.8 lumens/m^2 intensity incandescent lighting.

Significant decreases in light reflectance at 474, 525, 538, 568, and 571 nm and R474/R525 nm ratio were noted between 0 time and 5 min post cutting. Little other significant change was noted as steaks were allowed to bloom, except light reflectance at 600 to 630 nm increased continually until attainment of maximum bloom.

Light reflectance at 474, 538, 571 nm, R474/R525 nm, and R571/R525 nm tended to increase during development of Mb+ discoloration of beef longissimus dorsi steaks. Reflectance at 600, 610, 620 and 630 nm decreased gradually during this same change.

Reflectance at wavelengths and ratios studied generally was not greatly different for fresh steaks (predominantly reduced Mb) and discolored steaks (predominantly Mb+) except at 630 nm.

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CHAPTER 4

THE EFFECT OF SUBJECTIVE MATURITY AND MARBLING ON COLOR STABILITY OF FRESH PREPACKAGED BOVINE LONGISSIMUS DORSI MUSCLE

INTRODUCTION

Marbling, maturity, and muscle color as subjectively appraised in beef carcasses are determining factors of U.S.D.A. beef quality grades, and as a result affect carcass market value. Much research deals with the relationship of these factors to eating quality. Limited research has examined how certain beef quality factors affect display life of beef cuts prepackaged in transparent film for retail merchandizing. Hunt (1970) found no significant difference in objective and subjective values between three marbling levels of frozen lamb chops evaluated during a 44 day display period while in fresh, frozen, packaged, unpackaged, and thawed states. Romans et al. (1965) found higher Munsell hue data calculated from reflectance readings on moderate as compared to slight marbled beef steaks after the measured meat surface was exposed to room temperature air approximately 1 hr. Also, they found higher Munsell value means in A maturity beef steaks as compared to B, C, and D maturity levels. Birmingham et al. (1966) demonstrated color degradation was not necessarily associated with firmness in beef steaks cut from firm and soft U.S.D.A. Choice and Good beef short loins.

Romans et al. (1965) modified Fleming's et al. (1960) method slightly in assaying pigments from 80 beef rib steaks of different maturity and marbling levels and found the younger group examined had significantly ($P < .01$) less myoglobin (Mb) while no difference existed in Mb between slight and moderate marbling groups. Also, this group measured light reflectance on the cut surface of steaks and discovered highly significant ($P < .01$) but low correlations

between Munsell value and Mb content.

This project was designed to determine the influence of beef maturity and marbling on color brightening and subsequent discoloration as measured visually and by reflectance spectrophotometry.

EXPERIMENTAL PROCEDURE

Longissimus dorsi samples from the left wholesale rib cut of 60 steer carcasses (241 to 438 kg) were studied. Carcasses of unknown history were selected in commercial packing plants at 24 hr postmortem to represent both small and moderate marbling levels within each of three U.S.D.A. physiological age groups; A-A, A+B-, and B B+ (referred to as young, intermediate, and approaching maximum maturity, respectively). Degree of maturity was determined by the amount of bone ossification and color of lean as defined by Beef Carcass Grade Standards (U.S.D.A., 1965). Color of lean was only used as a "sample elimination factor" if it did not conform to the color typical for a maturity group. "Dark cutting" carcasses were not included in this study. Ten carcasses were selected per experimental cell.

The wholesale ribs were transported to Kansas State University meat laboratory and cut at 10 days postmortem. A rib steak approximately 5.08 cm thick was removed over the 8th thoracic vertebra. The longissimus dorsi muscle was separated; cut into two 2.54 cm thick portions immediately before the first objective color reading.

A freshly cut surface of the longissimus dorsi was used for subjective and objective color measurements. This sample was wrapped in cellophane, oxygen permeable when wet, with an opaque fiber board backing and sealed. The packaging film was approximately 0.028 mm thick, with about 0.006 mm low density polyethylene on a base sheet of cellophane. Fresh steaks were stored

in approximately 860.8 lumens/m^2 of incandescent light at about 6 C.

Both subjective and objective color measures (figures 2-10 and Appendix tables 1 and 2) were made at 26 time intervals. The 26 time intervals were: immediately after cutting; 5, 10, 15, 20 min; and 1, 2, 3, 10, 24, 48, 72, 96, 128, 136, 149, 154, 158, 163, 173, 178, 182, 187, 192, 216, and 240 hr post cutting.

Subjective color values (figure 2) were estimated in about 484.2 lumens/m^2 of cool white fluorescent light and on a white cotton background. Subjective definitions in this study were typical purplish red Mb (visual value 2.0), typical bright red oxymyoglobin (MbO_2, visual value 5.0), and typical flat or satin brown metmyoglobin (Mb+, visual value 9.0).

Color reflectance was recorded in the visible light range of 400 to 700 namometers (nm) on a Bausch and Lomb 600 reflectance spectrophotometer at scan speed of 250 nm/min using a MgO block wrapped in fresh meat cellophane for 100% reflectance. Care was taken to scan the same portion of sample surfaces each time and to exclude large fat or connective tissue areas from the sampled area. Duplicate reflectance scans were not recorded as color changes during the early time intervals were too rapid. Determination of pH at three loci (medial, central, and lateral) on the anterior steak surface adjacent to that used for reflectance percent was completed by surface probe. Samples for Mb determination were tightly wrapped in polyethylene coated locker paper, blast frozen at approximately -9 C about 24 hr, and held at -18 C. After 30 to 60 days of frozen storage, <u>longissimus dorsi</u> samples were removed from the storage freezer and allowed to warm-up a maximum of 45 min at ambient room temperature. Myoglobin was extracted and determined according to the method of Romans (1964) using a 17,000 equivalent molecular weight of pigments and constant hemoglobin molar absorptivity values (see Appendix

table 4 for details).

Percent transmittance from a Beckman DU spectrophotometer was determined on duplicate solutions of carbon monoxymyoglobin (MbCO, 95% agreement maintained) and also on the Bausch and Lomb 600 spectrophotometer using the same MbCO duplicate solutions (95% agreement maintained).

Additionally, on all reflectance values, in an attempt to simulate Snyder's (1965) adjusted R_A spectral values, the reflectance percent recorded at 525 nm was subtracted from each raw reflectance parameter. After subtracting the 525 nm percent, the numerical value of 10.0 was added to each and these new parameters were given the designation 525 adjusted or corrected reflectance percentages. Absorbance (A) logarithmic functions were computed from every raw reflectance and 525 adjusted reflectance percent parameters.

Analysis of variance and least significant difference (LSD) procedures were used to detect differences between marbling and maturity groups for all factors studied.

RESULTS AND DISCUSSION

Moderate marbled steaks had a significantly lower ($P < .01$) visual color score (table 1). These means include all pigment states and a higher score could mean faster oxygenation or faster deterioration. However, more color evaluations were done when color was deteriorating so a lower value probably is more indicative of slower deterioration. Figure 9 shows the mean visual color scores of two marbling levels plotted against time. Moderate marbled steaks had a slight visual color advantage at most time periods. They appeared brighter (higher score) at times up to 1 hr. After 24 hr, they showed a lower visual color mean which was indicative of less color deterioration. However, no significant interactions between time and marbling occurred for visual

Table 1. Marbling and maturity effects on mean visual color score and percent reflectance of bovine longissimus dorsi at 26 post cutting times.

	Visual score- m	Percent reflectance, nm									Reflect. Ratio	
		474	525	538	568	571	600	610	620	630	474/525	571/525
Marbling[n]												
Sm	6.88**	11.93	10.46*	8.31**	9.48**	9.33**	18.58	22.86	24.78	26.03	1.143**	0.886**
Me	6.76	11.93	10.70	8.60	9.98	9.81	18.96	23.13	24.92	26.00	1.117	0.910
Maturity												
A-A	6.85[b]	11.90[b]	10.57[b]	8.53[b]	9.83[b]	9.70[b]	18.60[a]	22.83[a]	24.71[a]	25.92[a]	1.124[a]	0.912[b]
A+B-	6.72[a]	11.58[a]	10.26[a]	8.12[a]	9.34[a]	9.15[a]	18.40[a]	22.49[a]	24.25[a]	25.42[a]	1.131[a]	0.883[a]
B B+	6.89[b]	12.31[c]	10.91[c]	8.71[b]	10.02[b]	9.87[b]	19.30[b]	23.67[b]	25.58[b]	26.69[b]	1.135[a]	0.899[ab]
Maturity X marbling												
Sm A-A	6.98[c]	12.71[c]	11.00[c]	8.86[d]	9.98[b]	9.85[b]	19.16[b]	23.53[b]	25.55[b]	26.92[b]	1.155[d]	0.886[ab]
Sm A+B-	6.62[a]	11.88[b]	10.59[b]	8.34[c]	9.65[b]	9.47[b]	19.02[b]	23.19[b]	24.97[b]	26.11[b]	1.123[b]	0.886[ab]
Sm B B+	7.04[c]	11.18[a]	9.78[a]	7.71[a]	8.81[a]	8.68[a]	17.56[a]	21.84[a]	23.80[a]	25.05[a]	1.150[cd]	0.887[ab]
Me A-A	6.72[ab]	11.08[a]	10.13[a]	8.20[b]	9.68[b]	9.55[b]	18.04[a]	22.12[a]	23.87[a]	24.93[a]	1.094[a]	0.939[c]
Me A+B-	6.82[b]	11.27[a]	9.93[a]	7.90[ab]	9.02[a]	8.83[a]	17.78[a]	21.79[a]	23.54[a]	24.72[a]	1.139[c]	0.880[a]
Me B B+	6.74[ab]	13.44[d]	12.04[d]	9.70[e]	11.24[c]	11.06[c]	21.04[c]	25.49[c]	27.36[c]	28.34[c]	1.120[b]	0.911[b]

a-e Means with same letter not significantly different ($P < .05$)

m Visual score: 2 = reduced Mb, 5 = MbO_2, 9 = Mb+

n Marbling: Sm = small, Me = moderate

*$P < .05$, **$P < .01$

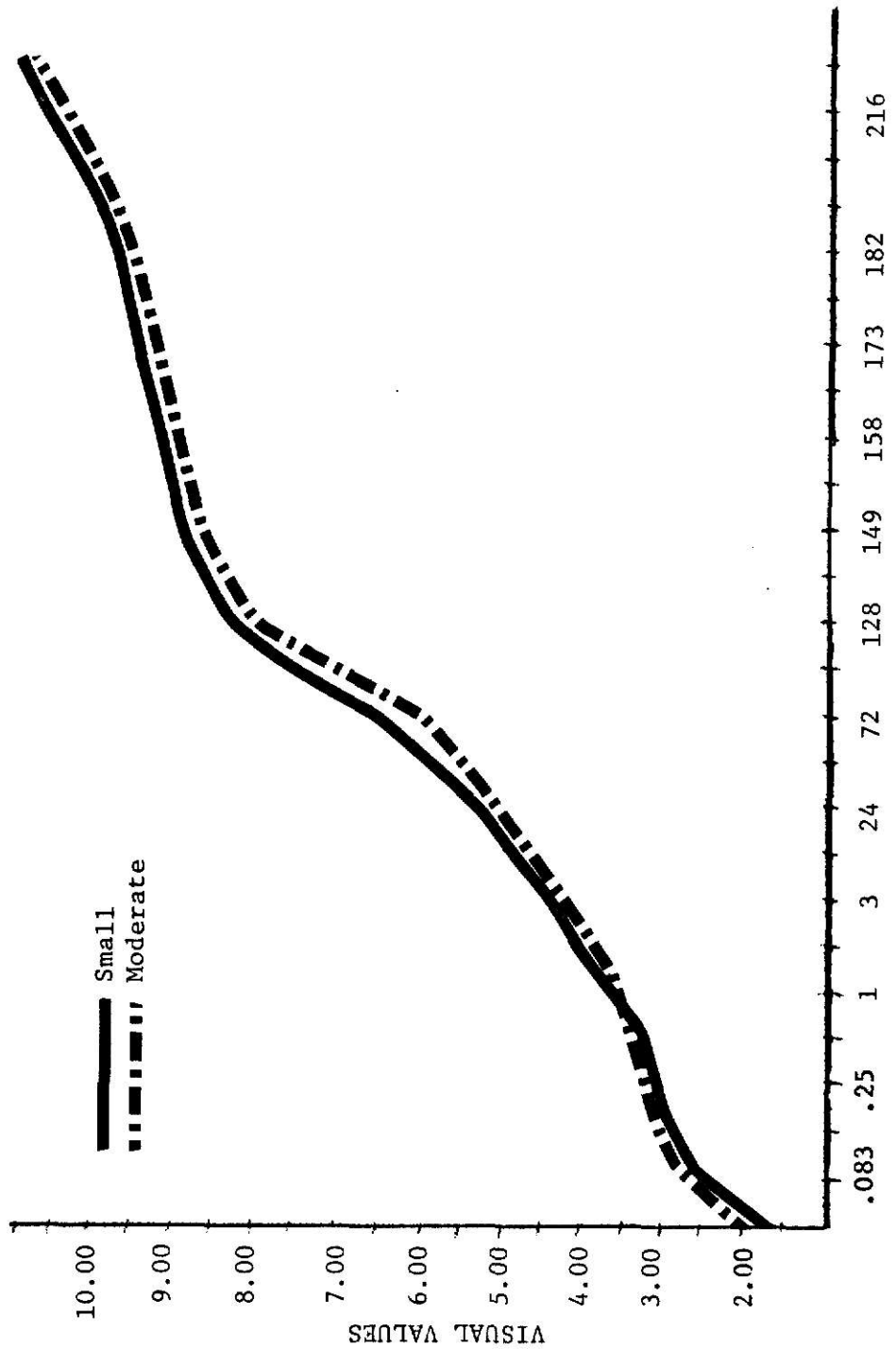

Fig 9. Subjective visual value of small and moderate marbled bovine longissimus dorsi at 26 post cutting times.

score, percent reflectance at nine wavelengths, and two reflectance ratios studied. Consequently, the marbling and maturity levels studied did not markedly enhance desirable color retention of fresh prepackaged beef longissimus dorsi kept in the conditions described. Reflectance percentages at 525, 538, 568, and 571 nm show significantly ($P < .05$) greater reflectance in the moderate marbled samples. Greater reflectance at 600, 610, and 620 nm may indicate brighter lighter red color. It may also result from less pigment according to Pirko and Ayres (1957) but no significant Mb difference was noted in this study between marbling levels (table 2). Romans et al. (1965) also found no difference between moderate and slight marbling groups. Ratio R474/R525 nm value of 1.117 for steaks with moderate marbling was significantly ($P < .01$) lower than for small marbling. This lower ratio supports the visual findings. Ratio R571/R525 nm mean for moderate marbling of 0.910 is significantly ($P < .01$) greater than for small marbling and is indicative of the trend noted visually. If one can assume more nearly equal values at 474, 525, and 571 nm are the result of flatter, straighter reflectance curves with less variation, then according to Weiner (1965) perhaps moderate marbled steaks are reflecting a different color hue as compared to small marbled samples. This would agree with results of Romans et al. (1965).

Marbling effects on adjusted A (table 2) were somewhat different than raw reflectance at wavelengths immediately adjacent to 525 nm, but otherwise similar. Only at 474 nm did this adjustment procedure appear to remove the effects of light scatter due to intramuscular fat.

The intermediate maturity group (A+B-) had the lowest (most desirable) visual mean score (table 1), but significantly ($P < .05$) less reflectance was noted in the intermediate maturity group than approaching maximum maturity group at all nine wavelengths. At 600, 610, 620, and 630 nm there was no

Table 2. Marbling and maturity effects on adjusted absorbance, myoglobin (Mb) concentration and pH of bovine longissimus dorsi at 26 post cutting times.

	Adjusted absorbance, nm								Mb	
	474	538	568	571	600	610	620	630	mg/gm	pH
Marbling[e]										
Sm	0.943**	1.115	1.056**	1.064**	0.750	0.662	0.629	0.612	4.39	5.62
Me	0.952	1.112	1.045	1.054	0.746	0.660	0.630	0.616	4.39	5.57
Maturity										
A-A	0.949[a]	1.109[a]	1.046[a]	1.054[a]	0.751[a]	0.664[b]	0.632[ab]	0.617[a]	4.13[a]	5.60
A+B-	0.949[a]	1.113[a]	1.052[a]	1.063[a]	0.750[a]	0.664[b]	0.634[b]	0.618[a]	4.41[ab]	5.62
B B+	0.946[a]	1.118[a]	1.053[a]	1.062[a]	0.744[a]	0.655[a]	0.623[a]	0.609[a]	4.63[b]	5.57
Maturity X marbling										
Sm A-A	0.935[a]	1.116[bc]	1.060[b]	1.068[b]	0.750[bc]	0.660[bc]	0.626[b]	0.608[ab]	3.84[a]	5.62
Sm A+B-	0.950[b]	1.120[cd]	1.054[b]	1.064[b]	0.742[b]	0.657[b]	0.626[b]	0.611[abc]	4.53[b]	5.66
Sm B B+	0.946[b]	1.108[ab]	1.054[b]	1.061[b]	0.759[d]	0.669[bc]	0.635[bc]	0.618[bcd]	4.82[b]	5.57
Me A-A	0.963[c]	1.102[a]	1.032[a]	1.040[a]	0.753[cd]	0.668[bc]	0.639[c]	0.626[a]	4.42[b]	5.57
Me A+A-	0.948[b]	1.106[ab]	1.050[b]	1.062[b]	0.757[cd]	0.672[c]	0.641[c]	0.624[cd]	4.30[ab]	5.59
Me B B+	0.946[b]	1.128[d]	1.052[b]	1.062[b]	0.729[a]	0.641[a]	0.611[a]	0.599[a]	4.44[b]	5.55

a-d Means with same letter not significantly different ($P < .05$)

e Marbling: Sm = small, Me = moderate

*$P < .05$, **$P < .01$

significant difference in percent reflectance between young and intermediate samples but significant differences were noted at 474, 525, 538, 568, and 571 nm with lower reflectance for the intermediate maturity group. Greater reflectance in the BB+ maturity group appears to be in conflict to significantly ($P < .05$) greater Mb concentration.

Intermediate maturity fresh cellophane wrapped beef had a slight visual color advantage (lower score) at some time periods (figure 10) especially from 128 to 187 hr, although no significant time-maturity interaction was calculated.

These data on maturity effects seem to be in further disagreement with data reported by Romans et al. (1965) where he reported significantly ($P < .01$) higher Munsell values in the youngest maturity group studied (A vs B, C, and D). Covington et al. (1970) studied the same group of ribs and found ether extract values of the approaching maximum maturity group to be significantly ($P < .05$) higher than the younger groups. If one can assume greater marbling reflects more light; Covington et al. (1970) may have partially explained the greater reflectance of the older ribs in this study. Also, mean reflectance percentages reported include all pigment states with a large proportion of readings in partial Mb+ state.

Neither ratio R474/R525 or R571/R525 nm exhibited significant maturity effects on interaction of time with maturity. The young group had a significantly higher R571/R525 nm ratio ($P < .01$) than the intermediate group (table 1), a result that conflicts somewhat with visual score results.

Maturity effects on adjusted A, adjusted to remove the effects of light scatter due to marbling and other uncontrolled variables, showed consistently fewer differences when compared to raw reflectance data at all wavelengths evaluated. Adjusted A effects were not significantly affected at any wavelengths and ratios examined, except at 610 and 620 nm.

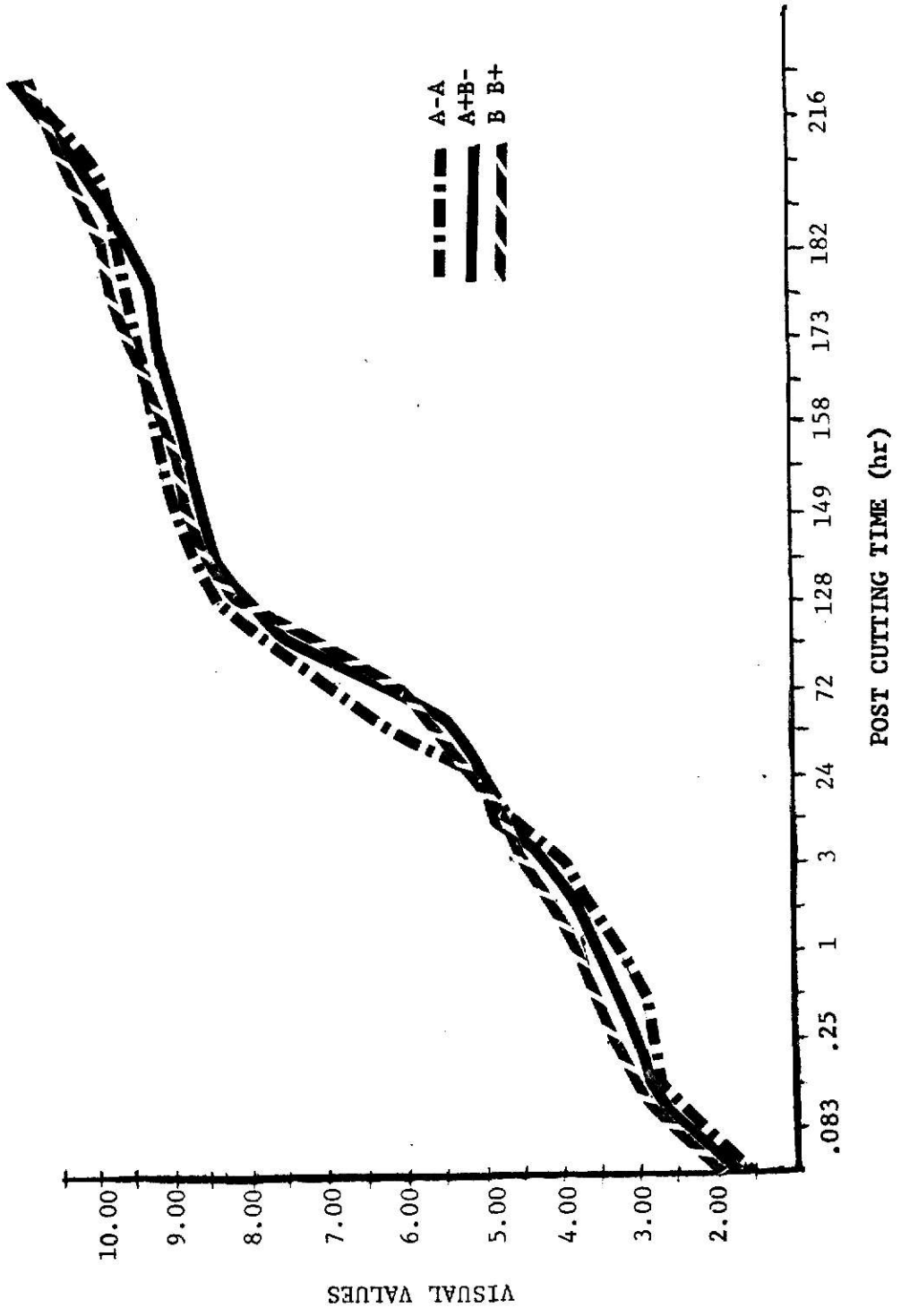

Fig 10. Subjective visual values of A-A, A+B-, and BB+ maturity levels of bovine longissimus dorsi at 26 post cutting times.

Marbling vs maturity interactions were highly significant ($P < .01$) for visual score, reflectance at all nine wavelengths, and both wavelength ratios studied (table 1). The moderate marbled BB+ (approaching maximum maturity) longissimus dorsi samples registered significantly ($P < .05$) higher reflectance percentages at all nine wavelengths recorded. The two more youthful groups with small marbling generally showed highest reflectance of the remaining groups for the nine wavelengths examined. Small A-A and small BB+ groups had significantly ($P < .05$) higher (less desirable) visual mean scores than other groups. Perhaps the divergence of this data lend support to Ledward's (1970) postulation that muscles with different fat contents likely cannot be compared by reflectance spectrophotometry.

Marbling and maturity interaction adjusted A effects were highly significant ($P < .01$) at all wavelengths and ratios studied as were raw reflectance percentages. On the basis of these data, in general, mathematical adjustments used here compared to spectral values, to eliminate uncontrolled variables and/or to obtain reproducible isobestic points, are not apparently necessary or advantageous to follow color changes of fresh bovine longissimus dorsi wrapped in poly coated cellophane fresh meat film. As has been pointed out by other workers, perhaps some procedure to help eliminate uncontrolled light scatter is necessary before reflectance can be used to compare various levels of marbling and maturity in fresh bovine longissimus dorsi.

The approaching maximum maturity (BB+) group in this study demonstrated significantly ($P < .05$) more Mb/gm of muscle tissue while the young group (A-A) had the least amount at 4.13 mg of Mb/gm of fresh frozen tissue (table 2). These results generally agree with results of Romans et al. (1965). No significant interactions were noted between instrument and marbling levels, instrument and maturity levels, and/or marbling and maturity. Muscle pH was

was not significantly affected by marbling or maturity variables in this study. Sample pH as measured by surface probe did correlate significantly ($P < .05$), although low, with mg of Mb/gm of tissue at -.25. No correlations of sample pH to reflectance data were calculated and the entire procedure deserves additional study.

SUMMARY

Cellophane wrapped fresh longissimus dorsi from the left wholesale rib cuts of 60 steer carcasses representing two marbling levels (small and moderate) and three maturity groups (A-A, A+B-, and BB+) were studied. Objective and subjective evaluations were made at 26 time intervals during display up to 240 hr under approximately 860.8 lumens/m^2 of incandescent light at about 6 C. Subjective values were one to eleven (increased as Mb changed to MbO_2 and to Mb+) and objective values were percent reflectance in the light range of 400 to 700 nm and two reflectance ratios.

Moderate marbled samples had a slight color advantage compared to small as they tended to brighten more rapidly after exposure to air and color deterioration was slightly slower. Intermediate maturity (A+B-) samples showed some superiority in visual score but the BB+ (approaching maximum maturity) steaks reflected more light at 474, 525, 600, 610, 620 and 630 nm than A-A (young) samples and more than A+B- (intermediate) at all wavelengths. No significant interactions between time and marbling and/or maturity occurred for visual score, reflectance at nine wavelengths, and two reflectance ratios studied. Maturity and marbling interactions were highly significant ($P < .01$) for visual score, reflectance at all nine wavelengths and the two wavelength ratios examined. The small young (A-A) and small old (BB+) samples had significantly ($P < .05$) poorer mean visual color as compared to the other

marbling maturity groups.

Except at 474 and 630 nm, the moderate marbled samples reflected the highest percent at wavelengths measured, perhaps due to greater ether extract. Only at 474 nm did the light scatter adjustment appear to improve the marbling effects presented. The incompatability of many of these data to earlier work seems at best to support Ledward's (1970) argument "it is unlikely that muscles with different fat contents can be compared by this technique."

The BB+ maturity group contained more Mb when compared to younger samples and no difference was noted between the A-A and A+B- groups. Myoglobin mg/gm was identical in the small and moderate levels of marbling. Surface probe pH was not significantly affected by maturity, marbling or their interactions.

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CHAPTER 5

THE RELATIONSHIP OF SPECTROPHOTOMETRIC REFLECTANCE TO VISUAL COLOR SCORE AND MYOGLOBIN CONCENTRATION

INTRODUCTION

Many workers, in making estimations of myoglobin (Mb) concentration and its derivatives through use of reflectance spectrophotometry, have studied various methods of reducing effects of uncontrolled variables such as intramuscular fat, wrapping film, depth penetrated by light beams, muscle texture and structure differences, pH and others (Broumand et al. 1958; Dean and Ball, 1960; Judd and Wyszecki, 1963; Ledward, 1970; Pirko and Ayres, 1957; Snyder, 1965; Snyder and Armstrong, 1967; Stewart et al. 1965). Pirko and Ayres (1957) studied the reflectance of U.S.D.A. Choice beef steaks stored for 14 days at approximately 6 C and packaged in various meat films. They determined reflectance at wavelengths of 635 nm (characteristic of metmyoglobin), 580 nm (characteristic of oxymyoglobin), and 555 nm (characteristic of Mb) to indicate changes in the respective Mb derivatives during display. Snyder (1965), while estimating quantities of the various Mb forms from reflectance spectra on the surface of U.S.D.A. Choice beef round steaks treated to contain predominantly Mb, oxymyoglobin (MbO_2) and metmyoglobin (Mb+), found too much scatter in the data and proceeded to make adjustments so that $R_A = 1.0$ at 525 nm. Snyder and Armstrong (1967) using earlier data collected by Snyder (1965) recommended that K/S (K = absorption coefficient, fraction of incident energy lost by absorption per unit of concentration per unit thickness of material and S = scattering coefficient, fraction of incident light lost due to scattering per unit thickness of material) values be utilized in attempts to relate reflectance data to the concentration of Mb derivatives in meat. Dean and Ball (1960) and Stewart et al. (1965) used K/S values to

quantify various forms of Mb. Ledward (1970) determined that standard deviations were less on minced beef semitendinosus samples of known pigment state when R_A was adjusted to one prior to calculating K/S values at 572 nm. He further stated, due to characteristic spectra of intramuscular fat, it is likely muscles with different fat contents cannot be studied accurately by reflectance spectrophotometry. Pirko and Ayres (1957) freed the pigment from 0.254 cm slices of U.S.D.A. Choice biceps femoris and semimembranosus by extraction with 2% KCl solution. They obtained much higher reflectances for pigment-extracted meat and postulated a low content of pigment would be a cause for the pale appearance of meat and for increasing spectral reflectance at all wavelengths.

Muscle Mb content has been measured in various ways, all time consuming. Fleming et al. (1960) using beef shank, rib eye, and sirloin tip muscles from five Hereford steers determined Mb quantitatively using four different methods: conversion of total pigments to cyanmet-compounds, conversion to carbon monoxide compounds, conversion to cyanmetmyoglobin after removal of hemoglobin, and pigments calculated as oxygenated compounds. They found the conversion of muscle pigments to carbon monoxide compounds provided the most favorable results.

In this study the relationship of objective reflectance information of fresh beef longissimus dorsi to visual color was followed. The effect of adjusting raw reflectance data to a constant value at 525 nm and calculating absorbance (A) was studied. The present study also explored usefulness of reflectance spectrophotometry for predicting Mb concentration.

EXPERIMENTAL PROCEDURE

Longissimus dorsi from the left wholesale rib cut of 60 steer carcasses

(241 to 438 kg) was studied. Carcasses of unknown history were selected in commercial packing plants at 24 hr postmortem to represent both small and moderate marbling levels within three U.S.D.A. physiological age groups; A-A, A+B-, and BB+ (young, intermediate and approaching maximum maturity). Degree of maturity was determined by the amount of bone ossification and color of lean as defined by the Beef Carcass Grade Standards (U.S.D.A., 1965). Color of lean was only used as a "sample elimination factor" if it did not conform to the color typical for a maturity group. "Dark cutting" carcasses were not included in this study. Ten carcasses were selected per experimental cell.

The wholesale ribs were stored for 10 days at approximately 2 to 4 C before cutting. The longissimus dorsi was taken from a 5.08 cm steak removed over the 8th thoracic vertebral location and cut into two 2.54 cm thick portions. The posterior portion was wrapped in fresh meat cellophane and its anterior surface was used for objective and subjective color measurements. The subjective values in this study ranged from 1 to 11 indicating the chemical state of Mb (figure 2); including typical purplish red Mb (visual value 2.0), typical bright red MbO_2 (visual value 5.0), and typical flat or brown Mb+ (visual value 9.0). Color reflectance was recorded in the visible light range of 400 to 700 nm on a Bausch and Lomb 600 spectrophotometer at a scan speed of 250 nm/min using a MgO block wrapped in cellophane for 100% reflectance.

Additionally, on all reflectance values, in an attempt to simulate Snyder's (1965) adjusted R_A spectral values, the reflectance percent recorded at 525 nm was subtracted from each raw reflectance parameter. After subtracting the 525 nm percent, the numerical value of 10.0 was added to each and these new parameters were given the designation 525 adjusted or corrected

reflectance percentages. Absorbance logarithmic functions were computed from every 525 adjusted reflectance percent parameter.

Samples for Mb determination were tightly wrapped in polyethylene coated locker paper, blast frozen at approximately -9 C about 24 hr and held at -18 C. After 30 to 60 days of frozen storage, longissimus dorsi samples were removed from the storage freezer and allowed to warm-up a maximum of 45 min at ambient room temperature. Myoglobin was extracted and determined according to the method of Romans (1964) using a 17,000 equivalent molecular weight for the pigments and constant hemoglobin (Hb) molar absorptivity values (see Appendix table 4 for details).

Percent transmittance from Beckman DU and Bausch and Lomb 600 spectrophotometers were determined on duplicate solutions of carbon monoxymyoglobin (MbCO, 95% agreement maintained).

Pooled correlation coefficients within each time period were calculated, removing effects of marbling and maturity, to determine the relationship of unadjusted reflectance percentages at the various wavelengths with subjective color scores and Mb concentration at each time period. Pooled correlation coefficients (removing effects of marbling, maturity and time) also were calculated.

RESULTS AND DISCUSSION

Highly significant ($P < .01$) pooled correlations, (marbling, maturity, and time effects removed) though low, were calculated between visual score and raw reflectance percentages at all but 525 nm of the 11 wavelengths and wavelength ratios in this project (table 3). The highest value, 0.33, occurred at the R474/R525 nm ratio. These correlations were positive for wavelengths from 474 to 571 nm indicating that at most time periods as visual value in-

Table 3. Pooled correlation coefficients between various methods of color-appraisal.

Wavelength	Visual score reflectance a	Visual score adjusted absorbance b
474	0.19 **	-.35 **
525	0.08 *	0.0
538	0.20 **	-.18 **
568	0.12 **	-.08 *
571	0.14 **	-.11 **
600	-.14 **	0.30 **
610	-.17 **	0.31 **
620	-.16 **	0.28 **
630	-.15 **	0.27 **
474/525	0.33 **	-.35 **
571/525	0.14 **	-.11 **

a Reflectance percent as recorded on percent transmittance scale.

b Logarithmic function of 525 adjusted reflectance; adjusted absorbance equals 2.0 - $\log_{10}$ x 525 adjusted reflectance percent.

* $P < .05$, ** $P < .01$.

creased (brightened and then deteriorated) a corresponding increase in percent of both unadjusted and adjusted reflectance occurred at these wavelengths. However, at the same time negative correlations were calculated between visual score and reflectance at 600, 610, 620, and 630 nm. It must be kept in mind that the subjective visual color scale used progressed from low values for Mb, to medium values for MbO_2, and high values for Mb+. One should look at correlation patterns with time periods (table 4).

When pooled correlations were calculated between visual scores and reflectance percent to remove effects of marbling and maturity at each of the 26 time periods, no significant relationships occurred at zero time (table 4). Nevertheless, from 5 min post cutting and prior to development of maximum bright red color at 3 hr post cutting, all but four of the relationships between reflectance at the nine wavelengths examined and visual score were highly significant ($P < .01$). All these significant correlations were positive and ranged from 0.38 to 0.67. As the steaks brightened in visual color (increased in value) between 5 min and 3 hr, raw reflectance percentages tended to increase at all nine wavelengths examined. At the period of maximum bright cherry red color, 24 hr post cutting, these correlations at all nine wavelengths and two ratios are low and negative but great homogeneity of sample color may have caused the low correlations. Visual scores correlated highly significant ($P < .01$) with raw reflectance (table 4) at 474 and 525 nm ($r =$ 0.48 or more) during early steak brightening, between 5 and 20 min post cutting, but then gradually became lower up to 3 hr and finally inconsistent.

Relationships between visual score and 538 nm reflectance were greatest at 216 and 240 hr and from 128 to 154 hr post cutting. Highly significant correlations ($P < .01$) of visual score vs raw reflectance at 568 and 571 nm were shown at early steak brightening ($r = 0.43$ to 0.54) at 128 and 136 hr

Table 4. Correlation coefficients of subjective visual score with reflectance data at various times post cutting.[a]

Time	Percent reflectance, nm									R474/R525	R571/R525
	474	525	538	568	571	600	610	620	630		
0	0.15	0.13	0.10	0.11	0.10	0.18	0.19	0.18	0.19	0.01	-.05
5 min	0.48	0.50	0.40	0.44	0.44	0.60	0.62	0.62	0.62	-.19	-.07
10 min	0.47	0.52	0.40	0.46	0.45	0.60	0.60	0.60	0.59	-.14	-.27
15 min	0.57	0.59	0.46	0.54	0.53	0.65	0.67	0.67	0.66	-.05	-.13
20 min	0.48	0.54	0.42	0.49	0.48	0.61	0.61	0.61	0.61	-.20	-.23
1 hr	0.29	0.48	0.31	0.44	0.43	0.64	0.66	0.67	0.65	-.35	-.05
2 hr	0.14	0.38	0.20	0.39	0.37	0.54	0.55	0.54	0.53	-.35	-.02
3 hr	0.01	0.28	0.08	0.28	0.26	0.49	0.48	0.45	0.44	-.45	0.02
10 hr	-.47	0.07	-.18	0.15	0.10	0.47	0.51	0.44	0.43	-.92	0.15
24 hr	-.20	-.17	-.17	-.16	-.17	-.14	-.12	-.14	-.15	-.17	-.02
48 hr	0.20	0.16	0.26	0.26	0.26	-.02	-.16	-.22	-.27	0.22	0.34
72 hr	0.04	-.06	0.23	0.26	0.28	-.46	-.64	-.69	-.72	0.46	0.63
96 hr	0.06	-.12	0.34	0.36	0.37	-.57	-.72	-.75	-.78	0.60	0.66
128 hr	0.31	0.12	0.51	0.49	0.50	-.46	-.65	-.68	-.70	0.64	0.65
136 hr	0.32	0.11	0.53	0.48	0.50	-.52	-.68	-.70	-.71	0.74	0.66
149 hr	0.39	0.14	0.51	0.40	0.42	-.51	-.61	-.62	-.62	0.78	0.51
154 hr	0.43	0.09	0.48	0.29	0.32	-.51	-.55	-.53	-.51	0.84	0.34
158 hr	0.30	-.03	0.32	0.11	0.14	-.52	-.52	-.47	-.44	0.85	0.18
163 hr	0.29	-.06	0.20	-.06	-.03	-.49	-.44	-.38	-.32	0.86	-.02
173 hr	0.24	-.09	0.06	-.22	-.19	-.48	-.40	-.29	-.21	0.81	-.22
178 hr	0.14	-.14	-.07	-.33	-.30	-.48	-.38	-.26	-.16	0.74	-.31
182 hr	0.24	-.07	-.09	-.38	-.36	-.41	-.25	-.08	0.03	0.76	-.46
187 hr	0.15	-.15	-.25	-.52	-.51	-.46	-.28	-.10	0.04	0.76	-.62
192 hr	0.12	-.18	-.34	-.58	-.46	-.48	-.24	-.04	0.13	0.75	-.50
216 hr	-.13	-.41	-.58	-.72	-.71	-.56	-.30	-.02	0.16	0.74	-.80
240 hr	-.02	-.32	-.50	-.68	-.68	-.51	-.24	0.06	0.23	0.78	-.80

r of 0.361 or greater, $P < .01$; r of 0.279 or greater, $P < .05$.

[a] Pooled correlation coefficients removing effects of marbling and maturity.

post cutting (r = 0.48 to 0.50), and during final four time periods (r = -.46 to -.72).

Wavelengths of 600, 610, 620, and 630 nm registered significant relationships to visual values at most time periods except 24, 48, and after 158 hr post cutting (table 4). Over 73% of these correlations were significant ($P < .05$) while over 69% were highly significant ($P < .01$), the latter values ranging from a low of -.38 to a high of -.78. From 5 min to 10 hr post cutting, a period of visual color brightening, reflectance percentages at 600, 610, 620, and 630 nm correlated highly significant ($P < .01$) and positive with visual score (table 4; range r = 0.43 to 0.67). Reflectance percent values at these same wavelengths had highly significant ($P < .01$) negative relationships with visual score between 72 and 158 hr post cutting (range r = -.44 to -.78). Reflectance percent at these wavelengths increased during visual steak color bloom and decreased as visual color deteriorated to brown. Relationships at 600 nm between visual score and reflectance percent were very encouraging as only zero time, 24 and 48 hr time periods of 26 failed to be highly significant ($P < .01$); ranging from r = -.41 to 0.65.

The highest correlation between an objective and visual value, -.92 (table 4), occurred at 10 hr post cutting with R474/R525 nm ratio as the objective variable. Other high relationships of 0.81 to 0.86 existed between this ratio and visual score between 154 and 173 hr post cutting. During further periods of visual color deterioration, between 72 and 240 hr post cutting, ratio R474/R525 nm correlated quite highly and significant ($P < .01$) with visual score. This would appear to substantiate advantages of this ratio in following color deterioration. Negative correlations were noted during the time of visual brightening when a higher visual score meant a brighter color, more representative of MbO_2. Positive and significant

correlations were calculated at 72 hr and later at which time higher visual scores may have indicated color deterioration. Therefore, one can conclude that darker color, whether due to Mb or Mb+ is associated with higher reflectance at 474 nm as compared to 525 nm.

Pooled correlation (removing effects of marbling, maturity, and time) coefficients between subjective (visual) and objective reflectance values in table 3 are low. Nevertheless, when the effects of time are removed from the relationships (table 4) higher and more favorable values were noted at 600, 610, 620, and 630 nm.

Correlation coefficients between visual score and adjusted A (table 3) were slightly improved at 474, 600, 610, 620, and 630 nm when compared to the correlation of visual score vs reflectance. Adjusted A values at 474, 600, 610, 620, and 630 nm were all less than unity and had a range of 0.469 to 0.99 A (Appendix table 3). Many values in other wavelengths were at one or greater (range 0.948 to 1.194). Adjusted A at 525 nm was calculated to be a constant value of one resulting in the correlation of zero with visual score.

Of the 26 time periods, visual values correlated (table 5) with Mb concentration on a highly significant level ($P < .01$) at only 5 min post cutting ($r = 0.37$). Other significant ($P < .05$) relationships between visual score and Mb concentration of -.32 and -.28 occurred at 20 and 15 min post cutting respectively. This indicates that a high Mb concentration tended to give visual impression of less complete conversion from Mb to MbO_2. Correlations of 474 nm reflectance percent and Mb concentrations at all time periods (table 5) were negative, therefore the higher reflectance values at 474 nm were indicative of less Mb. Furthermore, only six correlations of the 234 reported at nine different wavelengths and 26 time periods between reflectance and Mb concentration were positive. Romans et al. (1965) reported a

Table 5. Correlation coefficients of bovine longissimus dorsi myoglobin concentration with muscle reflectance measurements at various times post cutting. [a]

Time	Visual	Percent reflectance, nm									474/525	571/525
		474	525	538	568	571	600	610	620	630		
0	-.20	-.22	-.14	-.11	-.12	-.11	-.17	-.17	-.19	-.18	-.28	-.01
5 min	-.37	-.27	-.22	-.17	-.15	-.14	-.20	-.20	-.20	-.19	-.17	0.28
10 min	-.23	-.26	-.19	-.13	-.15	-.13	-.22	-.20	-.19	-.17	-.33	0.33
15 min	-.28	-.24	-.19	-.11	-.14	-.12	-.19	-.18	-.17	-.16	-.27	0.31
20 min	-.32	-.25	-.22	-.14	-.16	-.15	-.20	-.17	-.16	-.15	-.13	0.34
1 hr	-.24	-.15	-.11	-.02	-.04	-.04	-.09	-.08	-.08	-.08	-.10	0.23
2 hr	-.20	-.22	-.19	-.13	-.16	-.13	-.12	-.09	-.08	-.09	-.09	0.29
3 hr	-.27	-.19	-.14	-.07	0.08	-.08	-.11	-.08	-.08	-.09	-.11	0.26
10 hr	0.03	-.32	-.32	-.27	-.25	-.26	-.26	-.24	-.26	-.31	-.08	0.07
24 hr	0.05	-.48	-.41	-.37	-.31	-.32	-.41	-.45	-.48	-.58	-.35	0.22
48 hr	-.10	-.46	-.42	-.36	-.32	-.32	-.48	-.48	-.48	-.51	-.32	0.08
72 hr	0.05	-.38	-.34	-.27	-.25	-.25	-.30	-.24	-.24	-.25	-.29	-.08
96 hr	0.21	-.34	-.28	-.26	-.25	-.24	-.20	-.15	-.15	-.16	-.24	-.11
128 hr	0.14	-.23	-.18	-.10	-.08	-.07	-.20	-.20	-.22	-.23	-.20	0.07
136 hr	0.13	-.27	-.21	-.06	-.00	0.04	-.27	-.28	-.29	-.30	-.27	0.26
149 hr	0.02	-.29	-.24	-.06	0.01	0.04	-.23	-.25	-.28	-.30	-.21	0.32
154 hr	-.05	-.24	-.22	-.02	0.05	0.06	-.24	-.26	-.29	-.30	-.06	0.26
158 hr	-.04	-.30	-.27	-.12	-.02	-.00	-.21	-.21	-.26	-.28	-.06	0.27
163 hr	-.04	-.45	-.41	-.13	-.13	-.13	-.34	-.34	-.35	-.37	-.08	0.25
173 hr	0.03	-.46	-.42	-.22	-.11	-.10	-.34	-.34	-.38	-.42	-.05	0.35
178 hr	0.07	-.37	-.33	-.16	-.05	-.04	-.29	-.34	-.37	-.40	-.05	0.36
182 hr	0.03	-.42	-.38	-.25	-.16	-.15	-.30	-.35	-.38	-.39	-.10	0.23
187 hr	0.12	-.39	-.36	-.30	-.23	-.23	-.32	-.35	-.37	-.37	0.02	0.09
192 hr	0.22	-.47	-.45	-.39	-.30	-.25	-.38	-.41	-.42	-.39	-.02	0.04
216 hr	0.27	-.44	-.39	-.37	-.27	-.27	-.32	-.33	-.32	-.30	-.03	-.04
240 hr	0.14	-.47	-.40	-.34	-.27	-.27	-.32	-.37	-.40	-.40	-.05	0.01

[a] r of 0.361 or greater, $P < .01$; r of 0.279 or greater, $P < .05$.

significant ($P < .01$, $r = -.45$) relationship between Munsell value vs Mb content meaning when Munsell value increased (approached white which reflects more light), Mb concentration decreased. Therefore data presented here agree with Romans et al. (1965). Also, these data substantiate Pirko and Ayres (1957) postulation that low pigment content resulted in higher reflectance at all wavelengths. Reflectance at 474 nm had significant ($P < .05$) relationships with Mb concentration at 15 of 26 time periods with a tendency toward higher and more consistent relationships at 163 hr post cutting and later. This would tend to be in conflict with findings of Snyder (1965) who reported 474 nm to be a relatively stable and reproducible isobestic wavelength for Mb+ and MbO_2.

Relationships between reflectance at 525 nm and Mb concentration (table 5) were highly significant ($P < .01$) at 24 and 48 hr and significant ($P < .05$) from 163 to 240 hr post cutting. Variation in this relationship between time periods was not expected since Stewart et al. (1965) and Snyder (1965) reported A at 525 nm to be isobestic (identical A indices) for Mb, MbO_2, and Mb+. Furthermore, it was expected that if 525 nm was isobestic for all three Mb forms, that this reflectance value would have strongest relation to Mb concentration, but such was not the case. Correlations between Mb concentration and reflectance percent at 538 and 568 nm were fairly low (only 6 and 3 significant correlations, respectively). Except three time periods at 568 nm, correlations at these wavelengths were all negative. The nine significant ($P < .05$) relationships at these wavelengths occurred at time periods when MbO_2 or Mb+ were expected. In general reflectance at these wavelengths was unresponsive to Mb concentration.

The smallest number of significant relationships between Mb mg/gm and percent reflectance (table 5) occurred at 571 nm.

Correlations of percent reflectance at 630 nm vs Mb concentration (table 5) were negative and significant ($P < .05$) at 15 of the 26 time periods reported. No significant relationships occurred at early time periods up to 10 hr *post cutting*. Typical reflectance scans (figure 1) showed higher percentages at the 600 wavelengths (600, 610, 620, and 630 nm) and all exhibit negative correlations with mg/gm of Mb (table 5). Consequently, one can surmise that a chemical state of Mb other than reduced is causing higher reflectance percent at these wavelengths.

Wavelength ratios R474/R525 and R571/R525 nm showed inconsistent correlation patterns with Mb concentration and do not appear to be useful in predicting Mb concentration.

When Mb concentration was further correlated with reflectance at various time periods, the 24 and 48 hr intervals each had significant ($P < .05$) values at all nine wavelengths. At the 48 hr time period, seven of the nine wavelengths had highly significant ($P < .01$) relationships. However, the highest correlation was 0.58, meaning that about 34% of the variation in percent reflectance at 630 nm and 24 hr post cutting could be accounted for by Mb concentration.

In general the 1, 2, and 3 hr post cutting time periods seem to correlate poorly between Mb mg/gm and unadjusted reflectance at all wavelengths and ratios studied as compared to other times.

The Bausch and Lomb instrument registered a 60 sample mean of 4.35 mg of Mb/gm of tissue while the Beckman DU had a mean of 4.43 (not significantly different). The two instruments had *positive correlations* on pooled transmittance data of $r = 0.86$ at 474 nm, $r = 0.93$ at 538 nm, $r = 0.95$ at 568 nm, and $r = 0.50$ on mg of Mb/gm computations. The Beckman DU transmitted significantly ($P < .05$) more light at 474, 538, and 568 nm as compared to the Bausch and Lomb 600.

SUMMARY

Cellophane wrapped fresh bovine longissimus dorsi from the left wholesale rib cuts of 60 steer carcasses representing two marbling levels (small and moderate) and three maturity groups (A-A, A+B-, and BB+) were used to make subjective and objective color evaluations at 26 time periods ranging from zero time to 240 hr post cutting under display conditions of 860.8 lumens/m^2 at 6 C. Adjacent samples were used in analyzing Mb concentration.

Highly significant ($P < .01$), though low, pooled correlations (marbling, maturity, and time effects removed) were computed between subjective (visual) and objective (reflectance) values at nine wavelengths (474 to 630 nm), wavelength ratio R474/R525 and R571/R525 nm. These were positive from 474 to 571 and negative at 600, 610, 620, and 630 nm. Pooled correlations between visual score and raw reflectance percent (removing effects of marbling and maturity at each of the 26 time periods) showed increasing correlations at all nine wavelengths as samples brightened in subjective color (values increased). Correlations at 600 nm were highly significant ($P < .01$) at 23 of the 26 time periods studied (range of r = -.41 to 0.65). Ratio R474/R525 exhibited the highest correlation of -.92 at 10 hr post cutting and other higher relationships substantiate use of this ratio in following fresh bovine muscle color deterioration.

Pooled correlation coefficients between muscle reflectance measurements and Mb concentration (computed to remove the effects of marbling and maturity at each of 26 time periods) generally were highest at 24 and 48 hr post cutting (period of maximum visual bloom). At 5 min post cutting, visual correlation was greatest at -.37 ($P < .01$). Higher reflectance at all wavelengths was associated with lower Mb content as only six of 234 correlations at 26 times and nine wavelengths were positive. In general reflectance at these wavelengths

and wavelength ratios studied were too unresponsive to Mb concentration, to use them as predictors of Mb concentration, without further refinement or adjustment.

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CHAPTER 6

GENERAL SUMMARY

Rib steaks (2.54 cm thick) from 8th thoracic vertebral location of 60 steer carcasses (10 per cell) representing all combinations of three maturity (A-A, A+B-, BB+) and two marbling (small, moderate) groups were used. Immediately after exposing muscle to air, steaks were wrapped in oxygen permeable cellophane, and zero time color reflectance determined. Steaks were continuously stored under approximately 861 lumens/$(m)^2$ of incandescent light at approximately 6.7 C. Color reflectance (uncorrected) and visual score were determined at 26 time intervals ranging from zero time through 240 hr. Adjacent steaks to those used for color evaluation were surface probed for pH at three loci (medial, central, and lateral), wrapped in air-tight locker paper, frozen at -9 C and held at -18 C for maximum of 60 days. Myoglobin (Mb) was quantitatively extracted according to carbon monoxide method of Romans (1964).

Mean visual scores followed expected pattern with some onset of color deterioration at 96 hr. Of uncorrected reflectance values, those at 474 nm most closely follow expected pattern with higher value at zero time, little change through 48 hr and gradual increase from 96 through 240 hr. A significant decrease in percent reflectance between zero time and 5 min was observed at 474, 525, 538, 568, 571, and also with ratio R474/R525 nm. Generally, reflectance values at 525, 538, 568, and 571 nm were insensitive to color deterioration. A gradual decrease in reflectance at 600, 610, 620, and 630 nm was noted as color deteriorated. Ratio of R474/R525 nm tended to decline as color brightened, increased as color deteriorated and seemed more useful in following meat color changes than ratio of R571/R525 nm.

Moderate marbled and A+B- maturity steaks were more desirable in mean visual color with no significant interactions of marbling or maturity with time. Moderate marbled BB+ steaks reflected significantly more light at all wavelengths, possibly because of higher ether extract content. Generally marbling and maturity levels studied did not affect color stability.

Significantly more Mb occurred in BB+ maturity samples than the A-A maturity group. Marbling levels exhibited identical Mb concentrations. Muscle pH was not significantly affected by either marbling or maturity.

Pooled correlation coefficients (removing effects of marbling, maturity, and time) between subjective (visual) and objective reflectance percentages were positive ($P < .01$) at wavelengths less than 600 nm and negative at 600, 610, 620, and 630 nm. Pooled correlations of visual score vs reflectance percentages within each time period (removing effects of marbling and maturity) showed positive correlations at wavelengths 600, 610, 620, and 630 during steak brightening with all but four of these being highly significant ($P < .01$). During visual color deterioration, from 72 to 158 hr post cutting (increasing visual values), correlations were negative and highly significantly ($P < .01$) with values ranging from -.44 to -.78.

Reflectance data from earlier work was correlated (marbling and maturity effects removed) at each of 26 time periods with Mb concentration. Visual values correlated with mg/gm Mb best at 5 min post cutting. Predominance of negative correlations between Mb concentration and reflectance percentages indicates higher reflectance values are related to lower Mb concentrations. The highest correlation ($r = -.58$) between Mb concentration and reflectance percentages occurred on 630 nm at 24 hr post cutting. At 525 nm significant but low correlations of Mb concentration vs reflectance percentages were noted

at 14 of 26 time periods. However, these correlations do not encourage use of reflectance in determining Mb concentration without further refinement or correction.

APPENDICES

Appendix Table 1. Percent reflectance means of 60 fresh bovine longissimus dorsi at 26 time intervals post cutting.

Time	Visual	474	525	538	568	571
0	1.748^{a}	14.26^{k}	10.77^{cdef}	8.06^{c}	7.99^{bcd}	7.87^{bcd}
5 min	2.51^{b}	11.01^{abcd}	9.60^{a}	6.41^{a}	7.02^{a}	6.82^{a}
10 min	2.95^{c}	10.53^{a}	9.69^{ab}	6.38^{a}	7.14^{ab}	6.92^{ab}
15 min	3.14^{cd}	10.28^{a}	9.67^{ab}	6.36^{a}	7.19^{ab}	6.94^{ab}
20 min	3.30^{d}	10.32^{a}	9.77^{ab}	6.37^{a}	7.24^{ab}	7.02^{ab}
1 hr	3.60^{e}	10.62^{a}	10.11^{abc}	6.75^{ab}	7.63^{abc}	7.37^{abc}
2 hr	3.95^{f}	10.80^{abc}	10.34^{abcde}	6.94^{ab}	7.86^{abcd}	7.61^{abcd}
3 hr	4.34^{g}	10.69^{a}	10.40^{bcdef}	7.04^{ab}	8.05^{bcd}	7.78^{abcd}
10 hr	4.82^{h}	10.75^{ab}	10.25^{abcd}	6.86^{ab}	7.94^{abcd}	7.67^{abcd}
24 hr	5.06^{h}	10.84^{abc}	10.60^{cdef}	7.04^{ab}	8.39^{cde}	8.11^{cd}
48 hr	5.72^{i}	11.02^{abcd}	10.77^{cdef}	7.26^{b}	8.75^{de}	8.42^{de}
72 hr	6.24^{j}	11.65^{cdef}	11.18^{f}	8.09^{c}	9.77^{fg}	9.43^{f}
96 hr	7.40^{k}	11.59^{bcde}	11.01^{def}	8.40^{cd}	10.31^{ghi}	10.06^{fgh}
128 hr	8.19^{l}	11.81^{defg}	11.06^{ef}	8.79^{cde}	10.82^{hij}	10.58^{ghi}
136 hr	8.51^{m}	11.87^{defg}	10.93^{def}	9.04^{de}	11.06^{ijkl}	10.94^{hijk}
149 hr	8.80^{n}	12.19^{efgh}	10.98^{def}	9.55^{efg}	11.69^{jklmn}	11.58^{jkl}
154 hr	8.91^{no}	12.38^{efgh}	10.98^{def}	9.84^{fg}	12.01^{lmn}	11.90^{kl}
158 hr	9.07^{o}	12.51^{fghi}	10.94^{def}	10.02^{fgh}	12.07^{mn}	11.97^{l}
163 hr	9.17^{op}	12.37^{efgh}	10.71^{cdef}	10.02^{fgh}	12.10^{mn}	12.03^{l}
173 hr	9.36^{pq}	12.63^{ghi}	10.78^{cdef}	10.24^{gh}	12.20^{mn}	12.17^{l}
178 hr	9.46^{q}	13.02^{hij}	11.05^{ef}	10.66^{h}	12.52^{n}	12.50^{l}
182 hr	9.58^{q}	12.66^{ghi}	10.58^{cdef}	10.19^{fgh}	11.81^{klmn}	11.82^{kl}
187 hr	9.84^{r}	12.92^{hi}	10.68^{cdef}	10.18^{fgh}	11.50^{jklm}	11.51^{ijkl}
192 hr	10.18^{s}	13.29^{ij}	10.71^{cdef}	10.13^{fgh}	10.98^{ijk}	10.83^{ghij}
216 hr	10.63^{t}	13.87^{jk}	10.78^{cdef}	9.82^{fg}	9.91^{fgh}	9.94^{fg}
240 hr	10.88^{t}	14.28^{k}	10.70^{cdef}	9.45^{ef}	9.06^{ef}	9.10^{ef}

Means with same superscript letter not significantly different ($P<0.05$).

Appendix Table 2. Percent reflectance means of 60 fresh bovine longissimus dorsi at 26 time intervals post cutting.

Time	600	610	620	630	R474/R525	R571/R525
0	15.65^{ab}	21.48^{cd}	26.09^{h}	29.01^{ij}	1.332^{n}	0.727^{abc}
5 min	16.28^{bcde}	22.58^{def}	27.21^{hi}	30.14^{jkm}	1.153^{gh}	0.707^{a}
10 min	17.09^{cdefg}	23.51^{efg}	28.08^{ij}	31.07^{kmn}	1.090^{de}	0.714^{ab}
15 min	17.57^{efgh}	24.06^{efgh}	28.49^{ij}	31.57^{kmn}	1.064^{cd}	0.716^{ab}
20 min	17.77^{fgh}	24.27^{fghi}	28.65^{ij}	31.80^{mn}	1.060^{bcd}	0.719^{ab}
1 hr	18.92^{hi}	25.32^{hij}	29.00^{ij}	32.42^{nop}	1.052^{abc}	0.727^{abc}
2 hr	19.64^{ij}	25.88^{ij}	29.11^{j}	32.58^{nop}	1.044^{abc}	0.735^{abc}
3 hr	20.39^{jk}	26.52^{jk}	29.43^{jk}	32.67^{nop}	1.029^{ab}	0.744^{abc}
10 hr	22.55^{mno}	29.13^{lm}	31.66^{l}	34.38^{p}	1.048^{abc}	0.745^{abc}
24 hr	23.31^{no}	29.83^{m}	31.96^{l}	33.88^{op}	1.023^{a}	0.764^{bc}
48 hr	23.46^{o}	29.49^{lm}	31.13^{kl}	32.38^{no}	1.024^{a}	0.779^{c}
72 hr	22.86^{mno}	27.98^{kl}	29.19^{j}	29.70^{jk}	1.042^{abc}	0.837^{d}
96 hr	21.98^{lmn}	26.34^{jk}	27.13^{hi}	27.20^{hi}	1.053^{abc}	0.908^{e}
128 hr	21.56^{klm}	25.23^{ghij}	25.78^{gh}	25.64^{gh}	1.069^{cd}	0.946^{ef}
136 hr	20.59^{jkl}	23.68^{efgh}	24.07^{fg}	23.83^{fg}	1.086^{de}	0.997^{fg}
149 hr	19.91^{ij}	22.43^{de}	22.75^{ef}	22.38^{ef}	1.111^{ef}	1.050^{gh}
154 hr	19.34^{ij}	21.63^{cd}	21.78^{de}	21.35^{de}	1.131^{fg}	1.080^{hij}
158 hr	18.51^{ghi}	20.56^{bc}	20.73^{cd}	20.28^{cd}	1.147^{gh}	1.100^{hij}
163 hr	17.74^{fgh}	19.55^{ab}	19.62^{abc}	19.17^{abc}	1.159^{ghi}	1.129^{ij}
173 hr	17.50^{defgh}	19.22^{ab}	19.36^{abc}	18.94^{abc}	1.178^{hij}	1.134^{j}
178 hr	17.41^{defg}	19.13^{ab}	19.33^{abc}	18.94^{abc}	1.185^{ij}	1.133^{ij}
182 hr	16.50^{bcdef}	18.13^{a}	18.42^{a}	18.14^{a}	1.200^{jk}	1.117^{ij}
187 hr	16.06^{bcd}	17.93^{a}	18.42^{a}	18.27^{ab}	1.219^{kl}	1.077^{hi}
192 hr	15.76^{abc}	17.94^{a}	18.75^{ab}	18.87^{abc}	1.246^{l}	1.007^{g}
216 hr	15.06^{ab}	17.97^{a}	19.57^{abc}	20.21^{bcd}	1.295^{m}	0.913^{e}
240 hr	14.56^{a}	18.10^{a}	20.40^{bcd}	21.60^{de}	1.343^{n}	0.847^{d}

Means with same superscript letter not significantly different ($P < 0.05$).

Appendix Table 3. Absorbance * at various wavelengths of 60 fresh bovine longissimus dorsi at 26 time periods post cutting.

Time	474	538	568	571	600	610	620	630
0	0.871^{a}	1.139^{h}	1.143^{l}	1.151^{i}	0.829^{mn}	0.686^{h}	0.599^{d}	0.552^{d}
5 min	0.943^{g}	1.170^{jk}	1.131^{kl}	1.143^{hi}	0.781^{ijk}	0.642^{fg}	0.562^{c}	0.518^{c}
10 min	0.965^{ij}	1.179^{jk}	1.130^{kl}	1.143^{hi}	0.762^{hi}	0.626^{ef}	0.550^{c}	0.506^{bc}
15 min	0.974^{ijkl}	1.179^{jk}	1.125^{jkl}	1.140^{hi}	0.750^{gh}	0.616^{de}	0.544^{bc}	0.500^{bc}
20 min	0.977^{jkl}	1.186^{jk}	1.129^{kl}	1.142^{hi}	0.748^{gh}	0.614^{de}	0.543^{bc}	0.498^{bc}
1 hr	0.981^{klm}	1.183^{jk}	1.126^{kl}	1.141^{hi}	0.730^{fg}	0.604^{cde}	0.544^{bc}	0.495^{abc}
2 hr	0.984^{lm}	1.187^{jk}	1.126^{kl}	1.141^{hi}	0.720^{ef}	0.598^{bcd}	0.546^{bc}	0.496^{abc}
3 hr	0.990^{m}	1.183^{jk}	1.119^{ijkl}	1.135^{hi}	0.704^{cde}	0.588^{bc}	0.542^{bc}	0.496^{abc}
10 hr	0.982^{klm}	1.184^{jk}	1.115^{ijk}	1.131^{hi}	0.656^{a}	0.543^{a}	0.506^{a}	0.469^{a}
24 hr	0.990^{m}	1.194^{k}	1.109^{ijk}	1.125^{hi}	0.646^{a}	0.536^{a}	0.505^{a}	0.479^{ab}
48 hr	0.989^{m}	1.191^{k}	1.100^{hij}	1.118^{h}	0.646^{a}	0.544^{a}	0.520^{ab}	0.505^{bc}
72 hr	0.981^{klm}	1.165^{ij}	1.071^{g}	1.089^{g}	0.667^{ab}	0.578^{b}	0.560^{c}	0.554^{d}
96 hr	0.977^{jkl}	1.141^{hi}	1.042^{f}	1.055^{ef}	0.683^{bc}	0.606^{cde}	0.595^{d}	0.596^{e}
128 hr	0.970^{ijk}	1.122^{gh}	1.022^{ef}	1.034^{de}	0.694^{cd}	0.627^{ef}	0.619^{d}	0.624^{e}
136 hr	0.962^{hi}	1.101^{g}	1.006^{de}	1.013^{cd}	0.712^{def}	0.654^{g}	0.649^{e}	0.656^{f}
149 hr	0.952^{h}	1.076^{f}	0.981^{bcd}	0.987^{bc}	0.729^{fg}	0.679^{h}	0.675^{ef}	0.685^{g}
154 hr	0.945^{g}	1.062^{ef}	0.969^{ab}	0.975^{ab}	0.742^{gh}	0.695^{hi}	0.694^{fg}	0.705^{gh}
158 hr	0.938^{g}	1.049^{cde}	0.962^{ab}	0.968^{ab}	0.760^{hi}	0.716^{ij}	0.714^{ghi}	0.725^{hij}
163 hr	0.934^{fg}	1.036^{abcd}	0.951^{a}	0.954^{a}	0.773^{ij}	0.732^{jk}	0.731^{hij}	0.743^{ijk}
173 hr	0.927^{ef}	1.029^{abc}	0.950^{a}	0.952^{a}	0.781^{ijk}	0.740^{jkl}	0.738^{ij}	0.749^{jk}
178 hr	0.923^{def}	1.022^{ab}	0.948^{a}	0.950^{a}	0.790^{jk}	0.748^{klm}	0.744^{j}	0.754^{k}
182 hr	0.919^{de}	1.020^{a}	0.956^{ab}	0.956^{a}	0.802^{kl}	0.759^{lm}	0.753^{j}	0.761^{k}
187 hr	0.914^{cd}	1.025^{abc}	0.973^{abc}	0.973^{ab}	0.816^{lm}	0.766^{m}	0.754^{j}	0.759^{k}
192 hr	0.902^{c}	1.028^{abc}	0.996^{cd}	1.006^{c}	0.825^{m}	0.766^{m}	0.747^{j}	0.746^{jk}
216 hr	0.884^{b}	1.045^{bcde}	1.045^{f}	1.044^{e}	0.847^{no}	0.766^{m}	0.729^{hij}	0.716^{hi}
240 hr	0.868^{a}	1.059^{def}	1.082^{gh}	1.080^{fg}	0.860^{o}	0.761^{lm}	0.708^{gh}	0.684^{fg}

Means with same letter superscript not different ($P < .05$).

* Values adjusted to constant at 525 nm.

Appendix Table 4. Pigment determination of fresh beef.

Extraction

1. Weigh out approximately 50 gm of finely chopped longissimus dorsi muscle being careful to omit large pieces of fat and/or connective tissue.
2. Homogenize the sample with 120 ml of cold N/100 acetate buffer solution for 3 min in a Virtis model 45 homogenizer with variable speed set on high.
3. Pour slurry into a 250 ml polyethylene bottle. Rinse homogenizer and homogenizer bowl with buffer and pour into bottle. Centrifuge at 3000 rpm for 15 min.
4. Filter supernatant through cotton wad into a 500 ml Erlenmeyer flask.
5. Re-extract the residue with 80 ml of the buffer solution (homogenize for 1½ min with variable speed set on high). Pour slurry into same poly 250 ml bottle, rinse homogenizer and then centrifuge for 15 min at 3000 rpm.
6. Filter the re-extracted supernatant through the cotton and into the same 500 ml flask as previously. Rinse the funnel and cotton.
7. Filter the extract through Whatman No. 3 filter paper into a tared 500 ml beaker. Rinse the Erlenmeyer flask. Rinse the funnel and filter paper. Weigh the extract to the nearest one-half gm and report the gm weight as volume assuming one ml or cc equals one gm.
8. Divide the weighed volume for duplicate transmittance readings.

Notes

1. The buffer solution is prepared as follows:
   a. Prepare N/10 acetic acid solution and hold at 2 C. (11.45 ml acetic acid filled to 2 l).
   b. Prepare N/10 sodium acetate solution and hold at 2 C. (16.408 gm sodium acetate filled to 2 l).
   c. When the N/100 buffer solution is needed, i.e., before each use, mix the above stock solution with distilled water in the following ratio: 10 ml N/10 acetic acid
      10 ml N/10 sodium acetate
      180 ml distilled water.

   The pH of the buffer is approximately 4.5.
2. Difficulty is frequently encountered in obtaining a perfectly clear solution of the cyanmet-pigments. Another solution will often have to be prepared. It has been observed that there is less difficulty with this problem if the solutions are prepared and read immediately after extraction. The solutions must be perfectly clear for spectrophotometric readings.

Myoglobin (carbon monoxide conversion) Quantitation

1. Pipette 50 ml of the extract into an Erlenmeyer flask containing 2.01 gm of $Na_2HPO_4 \cdot 7H_2O$ (Anhydrous Na_2HPO_4 = 1.055 gm).
2. Allow the solution to stand until all of the phosphate has dissolved.
3. Pour into a 40 ml centrifuge tube and centrifuge for 15 min at 3000 rpm.
4. Filter through Whatman No. 3 filter paper.
5. Rinse a Thunberg tube with the solution and pour in approximately 10 ml of the solution.
6. Evacuate the tube.

7. Bubble carbon monoxide through the solution for 10 min (about 2 bubbles/sec). Then add a pinch (about 100 mg) of $Na_2S_2O_4$. Bubble the carbon monoxide for 10 additional min.
8. Rinse a cuvette with a portion of the solution and quickly pour the solution into the cell. Fill the cell to the top and cover.
9. Immediately read the solution on a spectrophotometer at 538 and 568 nm against a blank of N/15 Na_2HPO_4 (slit width 0.10 mm and sensitivity 1).
10. With the cuvettes orientated so the light passes through the sample in the same direction as it did in the DU, obtain a transmittance scan on the Bausch and Lomb 600 spectrophotometer in the visible light range of 400 to 700 nm.
11. Duplicate transmittance readings had at least 95% agreement at 538 and 568 nm on each instrument (percent agreement equals smallest transmittance percent divided by largest transmittance percent times 100).

Notes:

1. The 2.01 (anhydrous 1.055) gm of $Na_2HPO_4 \cdot 7H_2O$ in 50 ml of solution makes a N/15 solution. This offsets the acidifying effect of $Na_2S_2O_4$.
2. Carbon monoxide is a poisonous gas which cannot be detected by odor. Therefore, it must be always used under a hood. Make sure exhaust fan is working. Extend the exhaust line from the side arm of the Thunberg tube upward under the hood while working. Always work with the window of the hood about half way down.
3. After operation, turn the valve on the carbon monoxide tank off. Then, with the exhaust fan still running, turn the valve on the pressure

gauge until all of the gas is expelled from it. Then close the pressure gauge valve by reversing the turn.

4. A slight headache is the first symptom of carbon monoxide poisoning. If this is noticed, turn off the gas and check the system for leaks. Also, check the exhaust of the hood. If due caution is taken, there is no danger from the gas; but carelessness cannot be tolerated.

5. Antidote for carbon monoxide poisoning is to get fresh air immediately and call for pulmotor; apply artificial respiration for at least 1 hr or until the pulmotor arrives. Administration of oxygen containing 5% carbon dioxide is beneficial; inhalation of ammonia or amyl nitrite is often of value.

6. Symbols: A = spectrophotometric Absorbance.

   e = molar absorptivity.

   Constant a (Ka) e^{Hb}_{538} = constant 14.8×10^3.

   Constant b (Kb) e^{Hb}_{568} = constant 14.5×10^3.

   Constant c (Kc) e^{Mb}_{538} = constant 14.8×10^3.

   Constant d (Kd) e^{Mb}_{568} = constant 11.8×10^3.

   Constant -39.96 = (11.8) (14.8) - (14.8) (14.5).

   Constant 17 = 17,000/1,000; 17,000 = the molecular weight of myoglobin; 1000 = conversion of mg to gm.

   Constant 5 = 5 ml of phosphate dilution.

7. Calculations:

$$\text{Myoglobin Carbon monoxide (MbCO)} = \frac{A\,568 \times Ka - A\,538 \times Kb}{Kd \times Ka - Kc \times Kb},$$

$$= \frac{A\,568 \times 14.8 - A\,538 \times 14.5}{11.8 \times 14.8 - 14.8 \times 14.5},$$

$$= \frac{A\,568 \times 14.8 - A\,538 \times 14.5}{-39.96}$$

$$\text{Mg myoglobin/gm of fresh frozen meat (mg Mb/gm)} = \frac{MbCO \times 17 \times (\text{ml volume of mixture} + 5\ \text{ml phosphate dilution})}{\text{weight of sample}}$$

8. The calculations are based on the modification of Poel's method.

LITERATURE CITED

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THE EFFECT OF SUBJECTIVE MATURITY AND MARBLING ON PREPACKAGED BOVINE LONGISSIMUS DORSI MUSCLE COLOR AS MEASURED BY VISIBLE REFLECTANCE SPECTROPHOTOMETRY

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

Food Science

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

Rib steaks (2.54 cm thick) from 8th thoracic vertebral location of 60 steer carcasses (10 per cell) representing all combinations of three maturity (A-A, A+B-, BB+) and two marbling (small, moderate) groups were used. Immediately after exposing muscle to air, steaks were wrapped in oxygen permeable cellophane, and zero time color reflectance determined. Steaks were continuously stored under approximately 861 lumens/$(m)^2$ of incandescent light at approximately 6.7 C. Color reflectance (uncorrected) and visual score were determined at 26 time intervals ranging from zero time through 240 hr. Adjacent steaks to those used for color evaluation were surface probed for pH at three loci (medial, central, and laterial), wrapped in airtight locker paper, frozen at -9 C and held at -18 C for maximum of 60 days. *Myoglobin* (Mb) was quantitatively extracted according to carbon monoxide method of Romans (1964).

Mean visual scores followed expected pattern with some onset of color deterioration at 96 hr. Of uncorrected reflectance values, those at 474 nm most closely follow expected pattern with higher value at zero time, little change through 48 hr and gradual increase from 96 through 240 hr. A significant decrease in percent reflectance between zero time and 5 min was observed at 474, 525, 538, 568, 571, and also with ratio R474/R525 nm. Generally, reflectance values at 525, 538, 568, and 571 nm were insensitive to color deterioration. A gradual decrease in reflectance at 600, 610, 620, and 630 nm was noted as color deteriorated. Ratio of R474/R525 nm tended to decline as color brightened, increased as color deteriorated and seemed more useful in following meat color changes than ratio of R571/R525 nm.

Moderate marbled and A+B- maturity steaks were more desirable in mean visual color with no significant interactions of marbling or maturity with time. Moderate marbled BB+ steaks reflected significantly more light at all

wavelengths, possibly because of higher ether extract content. Generally marbling and maturity levels studied did not affect color stability.

Significantly more Mb occurred in BB+ maturity samples than the A-A maturity group. Marbling levels exhibited identical Mb concentrations. Muscle pH was not significantly affected by either marbling or maturity.

Pooled correlation coefficients (removing effects of marbling, maturity, and time) between subjective (visual) and objective reflectance percentages were positive ($P < .01$) at wavelengths less than 600 nm and negative at 600, 610, 620, and 630 nm. Pooled correlations of visual score vs. reflectance percentages within each time period (removing effects of marbling and maturity) showed positive correlations at wavelengths 600, 610, 620, and 630 during steak brightening with all but four of these being highly significant ($P < .01$). During visual color deterioration, from 72 to 158 hr post cutting (increasing visual values), correlations were negative and highly significantly ($P < .01$) with values ranging from -.44 to -.78.

Reflectance data from earlier work was correlated (marbling and maturity effects removed) at each of 26 time periods with Mb concentration. Visual values correlated with mg/gm Mb best at 5 min post cutting. Predominance of negative correlations between Mb concentration and reflectance percentages indicates higher reflectance values are related to lower Mb concentrations. The highest correlation ($r = -.58$) between Mb concentration and reflectance percentages occurred on 630 nm at 24 hr. post cutting. At 525 nm significant but low correlations of Mb concentration vs. reflectance percentages were noted at 14 of 26 time periods. However, these correlations do not encourage use of reflectance in determining Mb concentration without further refinement or correction.