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EFFECTS OF FREEZING SYSTEM, FREEZING RATE, FILM PERMEABILITY,
PACKAGING TIME AND DISPLAY CASE TEMPERATURE ON
COLOR STABILITY AND WEIGHT LOSS OF FROZEN BEEF
LONGISSIMUS AND PSOAS MAJOR MUSCLES

by *682*

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TABLE OF CONTENTS

	Page
LIST OF TABLES	iv
LIST OF FIGURES	vi
LIST OF APPENDICES	vii
ACKNOWLEDGMENTS	ix
ORGANIZATION OF THE THESIS	x
CHAPTER	
I. GENERAL INTRODUCTION	1
II. REVIEW OF LITERATURE	3
Meat Color and its Importance	3
The Chemistry of Muscle Pigments	4
Methods of Color Measurement	9
Factors Affecting Freezing Rate and Color of Frozen Beef	14
Freezing rates, methods and drip losses	14
Display temperatures, storage time and frozen weight loss	18
Packaging materials	20
Display lighting	22
Literature Cited	24
III. EFFECTS OF FREEZING SYSTEM AND FREEZING RATE ON COLOR STABILITY OF BEEF STEAKS PACKAGED IN TRANSPARENT FILM	32
Introduction	32
Experimental Procedure - Trial I	33
Description of steaks and treatments	33
Temperature recording	33

CHAPTER	Page
Packaging	36
Lighting and case temperature	36
Color evaluation	36
Experimental Procedure - Trial II	37
Results and Discussion - Trial I	37
Results and Discussion - Trial II	47
Summary	56
References	58
 IV. EFFECTS OF FILM PERMEABILITY, FREEZING SYSTEM, PACKAGING TIME AND DISPLAY CASE TEMPERATURE ON COLOR STABILITY AND WEIGHT LOSS DURING DISPLAY OF FROZEN BEEF <u>LONGISSIMUS</u> AND <u>PSOAS MAJOR</u> MUSCLES PACKAGED IN <u>TRANSPARENT FILM</u>	59
Introduction	59
Experimental Procedure	61
Description of steaks and treatments	61
Freezing and temperature recording	61
Packaging	63
Display cases and lighting system	63
Color and weight loss recording	65
Results and Discussion	65
Summary	91
References	95
 V. GENERAL SUMMARY	96
APPENDICES	100

LIST OF TABLES

Table		Page
1.	Effect of various freezing treatments on visual redness scores	34
2.	Effect of various freezing treatments on visual bleach scores	38
3.	Effect of various freezing treatments on percent reflectance at 650 nm	41
4.	Effect of various freezing treatments on percent reflectance at 685 nm	42
5.	Effect of various freezing treatments on total reflectance (area 1)	44
6.	Effect of various freezing treatments on red reflectance (area 2)	45
7.	Simple correlation coefficients between visual redness and bleach scores and objective variables	46
8.	Effect of various freezing treatments on visual redness scores	48
9.	Effect of various freezing treatments on visual bleach scores	50
10.	Effect of various freezing treatments on percent reflectance at 650 nm	51
11.	Effect of various freezing treatments on percent reflectance at 685 nm	52
12.	Effect of various freezing treatments on total reflectance (area 1)	54
13.	Effect of various freezing treatments on red reflectance (area 2)	55
14.	Simple correlation coefficients between visual redness and bleach scores and objective variables	57
15.	The effect of length of display on product weight loss ...	67
16.	Main treatment effects on visual redness scores of frozen beef <u>longissimus</u>	68

Table	Page
17. Main treatment effects on visual redness scores of frozen beef <u>psaos major</u>	69
18. Main treatment effects on visual bleach scores of frozen beef <u>longissimus</u>	71
19. Main treatment effects on visual bleach scores of frozen beef <u>psaos major</u>	72
20. Interaction effects on visual redness scores of frozen beef <u>longissimus</u>	73
21. Interaction effects on visual redness scores of frozen beef <u>psaos major</u>	74
22. Main treatment effects on 685 nm reflectance percentages of frozen beef <u>longissimus</u>	77
23. Main treatment effects on 685 nm reflectance percentages of frozen beef <u>psaos major</u>	78
24. Main treatment effects on total reflectance (area 1) of frozen beef <u>longissimus</u>	80
25. Main treatment effects on total reflectance (area 1) of frozen beef <u>psaos major</u>	81
26. Main treatment effects on percent red reflectance (area 2) of frozen beef <u>longissimus</u>	83
27. Main treatment effects on percent red reflectance (area 2) of frozen beef <u>psaos major</u>	84
28. Main treatment effects on R474/R525 ratio of frozen beef <u>longissimus</u>	86
29. Main treatment effects on R474/R525 ratio of frozen beef <u>psaos major</u>	87
30. Simple correlation coefficients between visual redness scores and objective variables (<u>longissimus</u>)	89
31. Simple correlation coefficients between visual redness scores and objective variables (<u>psaos major</u>)	90
32. Simple correlation coefficients between visual bleach scores and objective variables (<u>longissimus</u>)	92
33. Simple correlation coefficients between visual bleach scores and objective variables (<u>psaos major</u>)	93

LIST OF FIGURES

Figure	Page
1. Placement of thermocouple leads for temperature recording	35
2. Experimental design	62
3. Placement of thermocouple leads for temperature recording	64

LIST OF APPENDICES

Appendix	Page
1A Main treatment effects on 474 nm reflectance percentages of frozen beef <u>longissimus</u>	101
1B Main treatment effects on 474 nm reflectance percentages of frozen beef <u>psaos major</u>	102
2A Main treatment effects on 525 nm reflectance percentages of frozen beef <u>longissimus</u>	103
2B Main treatment effects on 525 nm reflectance percentages of frozen beef <u>psaos major</u>	104
3A Main treatment effects on 572 nm reflectance percentages of frozen beef <u>longissimus</u>	105
3B Main treatment effects of 572 nm reflectance percentages of frozen beef <u>psaos major</u>	106
4A Main treatment effects of 610 nm reflectance percentages of frozen beef <u>longissimus</u>	107
4B Main treatment effects of 610 nm reflectance percentages of frozen beef <u>psaos major</u>	108
5A Main treatment effects of 650 nm reflectance percentages of frozen beef <u>longissimus</u>	109
5B Main treatment effects on 650 nm reflectance percentages of frozen beef <u>psaos major</u>	110
6A Main treatment effects on percent blue reflectance (area 3) of frozen beef <u>longissimus</u>	111
6B Main treatment effects on percent blue reflectance (area 3) of frozen beef <u>psaos major</u>	112
7A Main treatment effects on R572/R525 ratio of frozen beef <u>longissimus</u>	113
7B Main treatment effects on R572/R525 ratio of frozen beef <u>psaos major</u>	114
8 Main treatment effects on weights of frozen beef steaks ...	115

Appendix	Page
9A Interaction effects on 474 nm reflectance percentages of frozen beef <u>longissimus</u>	116
9B Interaction effects on 474 nm reflectance percentages of frozen beef <u>psaos major</u>	117
10A Interaction effects on 610 nm reflectance percentages of frozen beef <u>longissimus</u>	118
10B Interaction effects on 610 nm reflectance percentages of frozen beef <u>psaos major</u>	119
11A Interaction effects on 650 nm reflectance percentages of frozen beef <u>longissimus</u>	120
11B Interaction effects on 650 nm reflectance percentages of frozen beef <u>psaos major</u>	121
12A Interaction effects on R572/R525 ratio of frozen beef <u>longissimus</u>	122
12B Interaction effects on R572/R525 ratio of frozen beef <u>psaos major</u>	123

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ORGANIZATION OF THE THESIS

This thesis is presented as a series of chapters. Chapter I 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 II includes a comprehensive review of literature pertinent to all topics and subjects included in subsequent chapters.

The two 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 V is a summarization of the entire thesis.

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

CHAPTER I

GENERAL INTRODUCTION

Thirty-five billion pounds of red meat were produced in 1969 and it is anticipated that more than 36 billion pounds will be produced in 1970, a large percentage of which will be retailed on a fresh basis. Centralized fabricating and packaging of red meat would allow increased efficiency in the use of labor and equipment, inspection at all levels of production, improved sanitation, reduced transportation tonnage and improved handling properties of shipped product. A modern freezing and packaging operation would easily fit into such a plant by producing a product of acceptable quality and appearance. Freezing may extend the display life of red meat considerably.

Educating consumers in the purchasing of frozen meat appears to be a major task, due to the experience of years of buying fresh meat and because of the natural suspicion consumers have of marketing changes in traditional products. Therefore, the appearance of frozen meat will be an extremely important selling point and the more natural the color the more likely the product will be accepted.

Meat displayed in the frozen state has several advantages over cuts displayed fresh. Most researchers agree that fresh meat color remains stable for approximately 72 hours, with excessive myoglobin oxidation resulting in discoloration after that amount of time. At temperatures above freezing, displayed meat is also subject to bacterial discoloration and spoilage. Freezing and low temperature

storage has been shown to greatly reduce both bacterial spoilage and discoloration. Ramsbottom (1947) reported an increased storage life and color stability in steaks stored at -12.2°C when compared with steaks stored at -3.3°C . Acceptable color was maintained for 90 days in steaks stored at -12.2°C . Lawrie (1966) reported finding edible meat in mammoth carcasses frozen for 20,000 years in Northern Siberia.

Workers have encountered several problems in the freezing of red meat. Two toning within cuts, partial discoloration of muscles, ice crystal formation, desiccation and frost in the packages have all plagued researchers at various times. These problems must be resolved before the meat industry is assured of stable saleable product.

The purpose of this work was to study the effects of freezing system, freezing rate, packaging time, film permeability, and display case temperature on color stability and weight loss of frozen beef longissimus and psoas major muscles.

CHAPTER II

REVIEW OF LITERATURE

Meat Color and its Importance

All factors which influence the visual appearance of meat are rapidly assessed by a consumer and interpreted into a response: to buy or not to buy; to eat or not to eat (Mackinney, Little and Briner, 1966). Color makes up a large part of the overall visual appearance of a cut of meat so it probably is the single greatest appearance factor which determines whether or not that cut will be purchased. Hiner (1954) stated that color of a product has both a psychological and a real effect on a consumer. The psychological effect occurs since color causes an almost immediate positive or negative response and the real effect indicates quality, amount of time held, temperature of holding, and how the product was handled.

Judging color is part of our everyday life (Judd and Wyszecki, 1963). Birren (1963) agreed and stated that everything owns its own color. He found that bright "warm" colors of certain foods tend to stimulate the autonomic nervous system, which affects the digestive system, while soft "cool" colors of other foods tend to suppress it. In further testing, other animals and birds were shown to react in the same manner as humans, so color is a universal means of judging acceptance or rejection of food.

Francis (1963) stated that color in food is important to humans and that it can be divided into two general areas. The first deals

with the addition of coloring agents to various foods while the second deals with the natural pigments, such as myoglobin and hemoglobin, found in meats and their contribution to color. The former can be controlled, so as to present an appealing color, but are illegal to use in many areas. Natural pigment state and concentration is difficult to control due to inherent differences in muscles and animals, and because of the different "physical" characteristics of each animal at the time of slaughter. These include differences in age, sex, nutritional state, ante mortem handling and post mortem treatment all of which could have significant effects on lean meat color.

The importance of meat color was demonstrated by Naumann, McBee and Brady (1957) who said consumers consider two different preferences in their meat purchasing. One is a minimum visual appearance which must be present if the meat cut is to be bought while the other is palatability which is determined by the overall quality of the meat. Certainly consumers have few if any means of estimating the flavor, juiciness and tenderness of a cut of meat while it is in the showcase so they must base their selection on the other preference, visual appearance. Color, of course, makes up much of what the consumer sees while making this choice.

The Chemistry of Muscle Pigments

The chemistry of meat color primarily involves the chemistry of the pigment myoglobin (Giffey et al., 1960). Brooks (1929) noted that the amount of myoglobin in beef muscle, unlike hemoglobin, is independent of the degree of blood removal, but dependent on the type of muscle, age

and condition of the animal. Rickansrud and Henrickson (1967) supported these findings by reporting muscle differences accounted for 84% of the variation in bovine myoglobin while animal differences accounted for 14% of the variation. They reported that 80% of beef longissimus pigment was myoglobin while only 62% of beef psaos major muscle pigment was myoglobin. The remainder of the pigment in both muscles was assumed to be hemoglobin. Earlier research by Shenk, Hall and King (1934) indicated that myoglobin made up more than 90% of fresh beef respiratory pigment, while hemoglobin percentage averaged 3.9%. Fleming, Blumer and Craig (1960) found similar results and reported that beef longissimus contains 95 to 97% myoglobin and 3 to 5% hemoglobin. Ginger, Wilson and Schweigert (1954) and Schweigert (1954) determined that beef longissimus and round muscles contain between 2.26 and 5.41 mg (averaging 3.9 mg) myoglobin per gram of fresh tissue.

Schweigert (1956) stated that myoglobin is a conjugated protein molecule. It contains a heme moiety attached to the protein, globin, which functions to accept oxygen from hemoglobin, for use in oxidative energy yielding reactions within muscle cells. Giffie et al. (1960) added that the protein portion of the molecule has a molecular weight of 16,000 to 17,000 and the non peptide portion, the heme, contains both an iron atom and a porphyrin ring. It is the oxidative state of the iron atom and the position of the heme on the globin that determines what color the myoglobin molecule exhibits.

Myoglobin and hemoglobin are identical in their color chemistry (Fox, 1966), with the most important reactions being autoxidation (metmyoglobin formed), reactions with NO (nitrosyl hemochrome formed),

oxygenation (oxymyoglobin formed) and denaturation. He stated that although the reactions are essentially the same, the rates for the two pigments are different. When freshly cut, the surface of meat contains myoglobin in the purple-red reduced state (iron atom in the non oxygenated iron II state). Immediately after exposure to available oxygen, oxygenation of the myoglobin begins which is visually observed as the formation of a bright red color (iron atom in the oxygenated iron II state) while prolonged exposure results in the oxidation of myoglobin to brownish-red metmyoglobin (iron atom in the non oxygenated iron III state) (Grant, 1955). Cutaia and Ordal (1964), using reflectance spectrophotometry, found that after as little as five minutes bloom time, no reduced myoglobin could be found in ground beef.

Many researchers have shown that partial pressure of oxygen, ambient temperature, pH, and enzymatic activity all affect the rate of pigment reactions. Conont and Fieser (1924) established that one hydrogen equivalent of oxidizing agent was required for the conversion of one mole of reduced myoglobin to metmyoglobin, and one hydrogen equivalent of reducing agent was needed for the reverse. Further work by Conont and Fieser (1924) and studies by Neill and Hastings (1925) showed that oxidation of oxymyoglobin to metmyoglobin was encouraged by low partial pressures of oxygen. Oxidation was most complete at a partial pressure low enough to permit one half of the ferrous atoms to be in the deoxygenated state.

George and Stratmann (1952a, b) found that with pH, salt concentration and temperature held constant, the oxidation of reduced myoglobin to metmyoglobin was a first order reaction where no protein

denaturation occurred. They determined the maximum rate of oxidation of myoglobin to occur at partial oxygen pressures between 1 and 1.4 mm Hg but later showed that this maximum rate could be obtained at oxygen pressures up to 20 mm of Hg if the temperature and pH were altered. At oxygen pressures above 20 mm of Hg the oxidation rates leveled off to a constant value. Calculations showed that the reaction changing one mole of myoglobin to metmyoglobin used 2.5 moles of oxygen. Snyder and Ayres (1961) verified the oxidation reaction to be a first order type by finding that when the log-percent of oxymyoglobin was plotted against time, a straight line resulted.

Brown and Mebine (1969) suggested that only 0.25 mole of oxygen in oxymyoglobin is used for oxidation and that 0.75 mole is released. They reasoned that former results could have included errors due to interference by oxidation products of sodium hydrosulfite, a reducing agent, which was not removed from the reaction.

Grant (1955) identified the enzymes found in frozen and thawed meat 18 hours after slaughter. Succinic dehydrogenase, glycerophosphate dehydrogenase and cytochrome oxidase were found to be the only active enzymes present at this time. Urbin and Wilson (1961) reported a uniform use of oxygen during the first 15 hours post mortem through uptake by myoglobin, enzyme systems and a dissolving of oxygen in meat solutions. After 15 hours, however, most of the oxygen use by muscle was due to uptake by enzyme systems. These enzyme systems continually re-reduced metmyoglobin to reduced myoglobin (Stewart et al., 1965b; Watts et al., 1966; Saleh and Watts, 1968). Hutchins, Liu and Watts (1967) found large differences within muscle systems in regard to

their initial reducing activity at different temperatures. Attainment of 50% reduction of metmyoglobin in 30°C took less than one hour. At 9°C it took 7 hours and at 0°C, 48 hours.

Brooks (1931) stated that as the pH of blood decreased, the rate of hemoglobin oxidation increased proportionally to the hydrogen ion concentration. Brown and Mebine (1969) supported the early findings of Brooks (1931), finding a strong dependence of oxidation rates on muscle pH, with a linear relationship existing between pH 5.0 and pH 7.0 with higher rates at 5.0. Urbin and Wilson (1961) disagreed and stated that with increasing muscle pH (6.4 to 8.0) oxygen uptake also increased.

Snyder and Ayres (1961) and Brown and Dolev (1963a) found that temperature has a profound effect on oxidative reaction rates. They reported doubling the rates by increasing the temperature from 0°C to 4°C. Further studies by Brown and Dolev (1963b) attempted to show effects of temperatures below 0°C on oxidation rates. They found that all myoglobin solutions oxidized more slowly at -5°C than at 0°C but when held at -10°C the solutions were frozen and the oxidation rates increased. They concluded that this was due to physical changes involved in freezing, and probably involved adjustment of the physical proximity of the myoglobin and oxygen molecules to a distance more favorable for oxidation reactions to occur. Brown and Mebine (1969) studied temperature effects on oxidative reaction rates and showed 40 to 50 fold slower rates at -2°C than at +22°C.

Brissey (1963) studied factors which affect the stability of meat pigments. It was found that sanitation, temperature, desiccation, packaging material, freezing and lighting could affect discoloration.

Methods of Color Measurement

Pearson (1969) stated that the eye sees color but biases and personality differences make an objective method of color determination necessary. These objective measurements must, however, involve procedures that can be reconciled with visual color perception. The University of Wisconsin (1963) published color standards for fresh pork, thus helping to meet the need for such an objective scale in the pork industry.

Mackintosh (1932) described Munsell disk colorimetry as a practical method of measuring color in beef. He determined that a "score of 40 red units" were needed to reach the lower limit of color acceptability. Nickerson (1946) authored a handbook on the method of disk colorimetry and described many uses of the system. Hiner (1954) further explained the use of the three variables, namely, hue (the color of an object), value (the degree of lightness or darkness), and chroma (the color difference from gray). He stated that by using these variables, the color of any cut of meat could accurately be determined and recorded.

Clydesdale (1969) described the International Commission on Illumination's system of tristimulus values as one using the three primary colors (red (x), green (y) and blue (z)), because all other colors are obtainable by mixing them. Little and Mackinney (1969)

added that all color stimuli with the same tristimulus values, under defined conditions, match each other whether or not they have the same spectral distribution. Under defined conditions of lighting and viewing, only one combination of the three primary colors will match a given set of tristimulus values. They continued, stating that the physical state of a sample will affect the results obtained from this or any other method of color measurement. High correlations, ranging from .769 to .873, were found between tristimulus values and visual beef and pork muscle color scores by Ockerman and Cahill (1969). The Gardner method, another color matching system, also employs three color variables.

Spectrophotometric methods of following pigment changes have been widely used and, in general, involve destruction of sample material. Early work by Austin and Drabkin (1935) described a method of determining heme pigment concentration using absorbency, based on the fact each pigment has a characteristic, reproducible absorption curve when pH and concentration are held constant. Schweigert (1954), however, while studying isolated myoglobin fractions, found several disadvantages with the absorbency method, due to the need to extract pigments and thereby change the chemical state of the sample. Once extracted, samples were not able to be used for further research.

Much work has been done regarding the spectral absorbency distribution of myoglobin derivatives. Each chemical form of myoglobin has its own characteristic spectral curve with easily observable maxima and minima (Snyder, 1965). Shenk et al. (1934) reported that solutions of myoglobin examined by spectrophotometric methods produced curves similar

spectral curves produced by hemoglobin, but were displaced toward the red portion of the spectrum. They found that by using absorbance methods, hemoglobin and myoglobin produced maxima at 547 nm and 577 nm, maxima at 413 nm and 582 nm, respectively. A minimum for hemoglobin appeared at 412 nm whereas 564 nm was found to be a minimum for myoglobin. Bowen (1949) studied absorption curves with a Beckman DU Spectrophotometer and found peaks at 555 nm (reduced myoglobin), 544 nm and 582 nm (oxymyoglobin) and 630 nm (metmyoglobin). Minima were found at 480 nm, 510 nm and 564 nm, and 590 nm for the three pigment forms, respectively. Similar peaks were found by Schweigert (1956), Tappel and Maier (1957) and others, but perhaps more important were ratios worked out to distinguish between pigment states.

An isobestic point for all three myoglobin oxidation forms was found to be 525 nm whereas reduced myoglobin and oxymyoglobin had an isobestic point at 572 nm and oxymyoglobin and metmyoglobin had an isobestic point at 474 nm (Stewart *et al.*, 1965a; Snyder, 1965; Snyder and Armstrong, 1967). Further work by the aforementioned workers explained that the ratios R_{474}/R_{525} nm and R_{572}/R_{525} nm indicate changes from reduced myoglobin to oxymyoglobin and oxymyoglobin to metmyoglobin, respectively. These ratios could then be used to follow and measure pigment changes in beef, during a storage period.

Broumand, Ball and Steir (1958) used absorbency ratios in fresh beef studies and found relative concentrations of oxymyoglobin decreased nearly with time when meat (in contact with air) was held at room temperature for up to 75 minutes. Metmyoglobin concentrations increased rapidly after four days storage at low temperatures.

Dean and Ball (1960b) found low correlations between absorbance and reflectance methods for measuring the proportion of the myoglobin state. After analyzing fresh meat by both methods, they reported values of 22%, 50% and 20% compared to 50%, 30% and 10% for myoglobin, oxymyoglobin and metmyoglobin, respectively, for the two methods. They suggested that reflectance presented a more accurate picture of surface pigment concentration, because myoglobin could be converted from one chemical state to another during extraction (absorbency method). True proportions of actual myoglobin derivatives could therefore not practically be determined. Spectral reflectance is suited to in situ studies of pigment systems where extractive procedures are difficult or impossible, or where they would cause an unwanted change in the pigment (Naughton, Frodyma and Zeitlin, 1957). These workers, however, used plots of the log of absorbance instead of reflectance. They were certain that both the spectral pattern and the maxima and minima of heme derivatives would not change when absorbance, rather than reflectance is used. Hansen and Sereika (1969) used absorbance ratios (A_{582}/A_{525} and A_{630}/A_{525}) to measure percentages of the three pigment forms and determined fresh meat showed acceptable bloomed color only if a A_{582}/A_{525} ratio greater than 1.12 and a A_{630}/A_{525} ratio less than 0.55 was obtained.

Reflectance was observed to be a rapid objective method of measuring muscle color (Ockerman and Cahill, 1969). Correlations of .88 were found between visual panel scores and percent reflectance at 685 nm. Kraft and Ayres (1954) used reflectance wavelengths between 540 nm and 800 nm to estimate color in round steak, and Pirko and Ayres (1957) found absorbance maxima of KCl extracted pigment at 500 nm and 635 nm

Tappel and Maier (1957) found similar results, stating heme containing compounds have characteristic maxima and minima in the regions 500 to 600 nm and 400 to 425 nm. They further stated reflectance was useful because no extraction was necessary. Allen et al. (1969) used reflectance to measure color changes in unfrozen beef longissimus muscle over 10 days (240 hours). Percent reflectance at wavelengths of 474, 525, 538, and 571 nm decreased significantly between 0 time and 5 minutes. Wavelengths of 525, 538, 568 and 571 nm were fairly insensitive to color change, while reflectance at 600, 610, 620 and 630 nm decreased gradually as subjective discoloration occurred. The ratio of R₄₇₄/R₅₂₅ nm increased as color deteriorated while that of R₅₇₁/R₅₂₅ nm did not change with darkening or discoloration.

A further application of reflectance spectrophotometry was used by Dean and Ball (1960b) who calculated K/S values (K/S equals the absorption coefficient (K), divided by the light scattering coefficient (S) per unit of sample thickness) of the ratios 507/573 nm and 473/597 nm to estimate the relative amounts of each pigment present in vacuum pre-packaged beef. Their data were not sufficient to explain the discoloration patterns that occurred. Stewart et al. (1965a) reported a linear relationship between K/S₅₂₅/K/S₅₂₅ and percent total pigment. The plot of raw reflectance at R₅₇₂/R₅₂₅ nm and percent total pigment, however, yielded a non linear curve, so graphs could accurately show percentages of the three myoglobin derivatives.

Snyder (1965) suggested adjusting reflectance to a common level at 525 nm to eliminate the effects of uncontrollable variables including marbling and film wrinkles so graphs could accurately show percentages