VISUAL SELECTION OF HIGH AND LOW PIGMENT BEEF LOINS AS RELATED TO FROZEN DISPLAY COLOR UNIFORMITY BETWEEN LONGISSIMUS AND PSOAS MAJOR MUSCLES

by

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CHAPTER I

INTRODUCTION

A consumer judges which food items to purchase by several criteria, but an important consideration is color. Meat color indicates to the consumer such factors as display case age of cuts, handling conditions, and intrinsic animal differences which affect, or are presumed to affect, "eating qualities" (Pangborn, 1967; Bailey, 1968; Stanton, 1971; Darrah, 1971).

Fresh displayed meat often remains in a saleable color condition up to 72 hours (Naumann, 1968). Within the time period of acceptable color, the cut must be selected by the consumer, or the retail store owner will have an unsaleable fresh meat cut. When fresh meat cuts become unattractive, unsold meat is often frozen and offered for resale in this form. This practice has contributed to consumer skepticism about quality of frozen meats in retail stores. Yet, approximately 70 percent of all fresh meat purchased at retail is frozen by the consumer prior to consumption (Ramsbottom, 1971).

An alternative to fresh meat, with its limited saleable life, is meat frozen prior to display, thereby extending display case life up to 7 to 21 days. This longer saleable life gives the retail store manager more time to adjust amount of any specific meat cut displayed to consumer demand. Therefore, overstocking of a particular cut is less likely to occur. Frozen distribution, storage, and display seems desirable with central cutting of retail cuts.

Central meat cutting likely will be more widely used in the future. Some advantages of central cutting combined with frozen distribution and display are: reduced individual store costs for space and facilities, better utilization of by-products, higher cutting skill levels in a central plant, greater sanitation, more standardized cuts, greater marketing flexibility and reduction of transportation costs (Kropf, 1971). Frozen meat production has rapidly expanded from 45 million pounds in 1950 to 510 million pounds in 1972, an increase of 11 1/3 times in 22 years (Anonymous, 1973).

One main reason for consumer reluctance to purchase frozen meat cuts is the gradual development of a dark red or brown undesirable color. Discoloration is especially notable when a color contrast occurs between muscles in the same retail cut. This occurs frequently in frozen retail cuts packaged in skin tight film, especially when the cut contains two muscles such as longissimus and psoas major that differ widely in red/white muscle fiber ratio.

The purpose of this study was to explore color uniformity or non-uniformity in beef T-bone steaks (longissimus and psoas major muscles) in an attempt to establish selection criteria for cuts best suited for frozen display. The selection criterion used was a visual estimate of heme pigment level.

CHAPTER II

REVIEW OF LITERATURE

Chemistry of Meat Color

Chemistry of meat color involves primarily chemistry of the muscle protein myoglobin (Bodwell and McClain, 1971). Myoglobin is a conjugated protein, with heme attached to the protein globin (Schweigert, 1956). An iron atom and a large planar ring, the porphyrin, constitute the heme group (Lemberg and Legge, 1949). Three different side chains, methyl, vinyl, and propyl, are attached to the porphyrin ring. Visual color is largely dependent upon the oxidative state of the iron and the concentrations of myoglobin and hemoglobin. Hemoglobin has a structure similar as myoglobin.

When meat is freshly cut, iron is in the reduced (Mb) state and is purplish-red in color. Upon oxygenation the pigment is in oxymyoglobin (MbO_2) state or cherry red in color, with iron still in the ferrous (++) state. Prolonged exposure to oxygen causes oxidation of the myoglobin pigment to metmyoglobin (Met Mb). The color is dark red or brown, and the iron is in the ferric (+++) state. Therefore, in the presence of oxygen, myoglobin is converted to two different pigment MbO_2 (cherry red) or Met Mb (dark red or brown). Relative proportions of these two forms depend upon the partial pressure of oxygen, the formation of Met Mb being favored at low oxygen pressures, ranging from 4 to 25 mm (George and Stratmann, 1952; Bodwell and McClain, 1971).

Selection for Pigment Level

The amount of myoglobin in meat is independent of amount of blood removed at time of slaughter, but depends on type of muscle, age, and general condition of the animal (Brooks, 1929; Rickansrud and Henrickson, 1967). Shenk et al. (1934) reported that of total heme pigment in beef longissimus, 95 to 97 percent is myoglobin and 3 to 5 percent hemoglobin. Rickansrud and Henrickson (1967) reported that blood heme pigment, hemoglobin, constitutes 12 to 30 percent of the total pigment of the longissimus, psoas major, biceps femoris, and semitendinosus muscles of beef. Enfield (1968) indicated that before anyone begins a selection program for a certain muscle color in animals, he must identify the characteristics which influence color and determine if they are genetic or environmental factors. He indicated that average porcine muscle color heritability is about 0.3 (moderately heritable). While most selected traits are on one end or the other of the normal distribution curve for that factor, muscle color presents a special problem, in that most desirable color is not represented by either extreme. Therefore, elimination of undesirable is more important than selection for desirable color. Pease and Smith (1965) found that porcine color is moderately heritable and that animal selection for muscle color would result in a genetic change in average color scores. They also found differences in muscle color between porcine breeds. Jonsson (1963) found that Danish pigs had darker muscle color in winter than during summer.

Schafer (1972) reported that lower pigment of longissimus and psoas major resulted in more uniform color between muscles after freezing in a skin-tight Iolon package. He also stated that original visual color brightness of oxygenated steaks cannot be used as a selection criterion for steaks that will maintain color uniformity in the frozen packaged condition.

Color Deterioration and Autoxidation

Considerable interest has centered around autoxidation (self induced or catalized oxidation) of myoglobin or the other heme pigments.

Autoxidation of oxymyoglobin (MbO_2) is known to be influenced by various factors such as pH (George and Stratmann, 1954; Matsuura et al., 1962; Brown and Mebine, 1969); temperature (Matsuura et al., 1962; Brown and Mebine, 1969; Brown and Dolev, 1963a,b); partial pressure of oxygen (George and Stratmann, 1952; Matsuura et al., 1962; Brown and Mebine, 1969); metallic ions (Snyder and Skrdlant, 1966) and bacteria (Robach and Costilow, 1961).

Fat Deterioration and Its Relation to Color Deterioration

Tappel (1962) showed that hematin compounds, such as myoglobin, catalyze the oxidation of unsaturated lipids. The critical reaction in myoglobin-catalyzed lipid oxidation appears to be the catalytic decomposition of hydroperoxide into free radicals. The catalytic activity has been found to be completely dependent on the presence of iron (Tappel, 1962). Younathan and Watts (1959) concluded that the ferric form of pigment is the active catalyst of tissue lipid rancidity and demonstrated lipid oxidation in lean meat, indicating the involve-

ment of cellular lipids. In the same study the addition of adipose fat was reported to have little or no effect on lipid or myoglobin oxidation. Liu and Watts (1970) presented evidence showing that both heme and non heme iron function as catalysts of lipid oxidation in meats. Hornstein et al. (1961) reported that cellular lipids extracted from lean muscle contain two to four percent (on a fresh tissue basis) triglycerides and 0.8 to one percent phospholipids. The unsaturated fatty acids with two and three double bonds constituted 6.1 and 25.2% respectively of combined triglyceride and phospholipid fractions. Phospholipids were reported to contain 19.2% of unsaturated fatty acids with four or more double bonds. It is evident that cellular lipids are more unsaturated than adipose lipids and are therefore more liable to oxidation. Hutchins et al. (1967) studied the interrelations between myoglobin and lipid oxidation in stored ground beef. Significant positive correlations between metmyoglobin content and thiobarbituric acid values were reported. Younathan and Watts (1959) showed that the oxidized form of the pigment is the active catalyst in tissue lipid rancidity. The results of Ledford and Macfarlane (1971) showed that higher initial metmyoglobin concentrations have no effect on rate of lipid oxidation. Govindarajan (1973) found that linoleic acid, methyl linoleate, and trilinoleic increased myoglobin oxidation in ground beef during storage. This indicates that the oxidation of lipids may be an important factor favoring the production of oxidized pigment.

REFERENCES

Anonymous. 1973. Truck transportation revolutionizes marketing of nation's meat. Meat Processing. Davies Pub. Co., Chicago. 12:68.

Bailey, M. E. 1968. Importance of color to the meat industry. Proc. Meat Ind. Res. Conf., Am. Meat Inst. Found., Chicago.

Bodwell, C. E. and P. E. McClain. 1971. Chemistry of animal tissues. Protein. In Science of Meat and Meat Products. W. H. Freeman and Co., San Francisco.

Brooks, J. 1929. Postmortem formation of methemyoglobin in red muscle. J. Biochem. 23:1391.

Brown, W. D. and A. Dolev. 1963a. Autoxidation of beef and tuna oxymyoglobins. J. Food Sci. 28:207.

Brown, W. D. and A. Dolev. 1963b. Effect of freezing on autoxidation of oxymyoglobin solution. J. Food Sci. 28:211.

Brown, W. D. and L. B. Mebine. 1969. Autoxidation of oxymyoglobin. J. Biol. Chem. 244:6696.

Darrah, L. B. 1971. Food marketing. The Ronald Press Company. New York.

Enfield, F. D. 1968. Problems related to the genetic analysis of differences in meat color. Proc. Meat Ind. Res. Conf., Am. Meat Inst. Found., Chicago.

George, P. and C. J. Stratmann. 1954. The oxidation of myoglobin to metmyoglobin by oxygen. 3. Kinetic studies in the presence of carbon monoxide and at different hydrogen-ion concentrations with considerations regarding the stability of oxymyoglobin. J. Biochem. 57:568.

George, P. and C. J. Stratmann. 1952. The oxidation of myoglobin to metmyoglobin by oxygen. 2. The relation between first order rate constant and the partial pressure of oxygen. J. Biochem. 51:418.

Govindarajan, S. 1973. Ph.D. Thesis, University of Massachusetts, Amherst.

Hornstein, I., P. F. Crowe and M. J. Heimberg. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci. 26:581.

Hutchins, B. K., T. H. P. Liu and B. M. Watts. 1967. Effect of additives and refrigeration on reducing activity, metmyoglobin and malonaldehyde of raw ground beef. J. Food Sci. 32:214.

Jonsson, P. 1963. Danish pig progeny testing results. Z. Tierz. Zucht Biol. 78:205.

Kropf, D. H. 1971. Frozen meat-potential and problems. Proc. Recip. Meat Conf. 24:176.

Ledford, D. A. and J. J. Macfarlane. 1971. Some observations on myoglobin and lipid oxidation in frozen beef. J. Food Sci. 36:987.

Lemberg, R. and J. W. Legg. 1949. Hematin compounds and bile pigments. Interscience, New York.

Liu, H. and B. M. Watts. 1970. Catalysts of lipid peroxidation in meats. 3. Catalysts of oxidative rancidity in meats. J. Food Sci. 35:596.

Matsuura, F., K. Hashimoto, S. Kikawada and K. Yamaguchi. 1962. Studies on the autoxidation velocity of fish myoglobin. Bull. Japanese Soc. Sci. Fish. 28:210.

Naumann, H. D. 1968. Cutting and packaging of fresh meats. Proc. Meat Ind. Res. Conf. Am. Meat Inst. Found., Chicago.

Pangborn, R. M. 1967. Some aspects of chemoreception in human nutrition. In the Chemical Senses and Nutrition. The John Hopkins Press, Baltimore.

Pease, A. H. R. and C. Smith. 1965. A note on the heritability of muscle colour in pigs. An. Prod. 7:273.

Ramsbottom, J. M. 1971. Packaging. The Science of Meat and Meat Products. W. A. Freeman and Co. San Francisco.

Rickansrud, D. A. and R. L. Henrickson. 1967. Total pigments and myoglobin concentration in four bovine muscles. J. Food Sci. 32:57.

Robach, D. L. and R. N. Costilow. 1961. Role of bacteria in the oxidation of myoglobin. Applied Microbiol. 9:529.

Schafer, D. E. 1972. Histochemical and biochemical characterization of bovine longissimus and psoas major muscles as related to frozen display color variations. Ph.D. Dissertation. Kansas State University, Manhattan, Kansas.

Schweigert, B. S. 1956. Chemistry of meat pigments. Proc. 8th Res. Conf. Am. Meat Inst. Found., Chicago.

Shenk, J. H., J. L. Hall and H. H. King. 1934. Spectrophotometric characteristics of hemoglobins. I. Beef blood and muscle hemoglobins. J. Biol. Chem. 105:741.

Snyder, H. E. and H. B. Skrdlant. 1966. The influence of metallic ions on the autoxidation of oxymyoglobin. J. Food Sci. 31:468.

Stanton, W. J. 1971. Fundamentals of marketing. McGraw-Hill Book Company.

Tappel, A. F. 1962. Hematin compounds and lipoxidase as biocatalyst. Symposium of Foods, Lipids and Their Oxidation. Avi Publishing Co. Westport, Conn.

Younathan, M. T. and B. M. Watts. 1959. Relationship of meat pigments to lipid oxidation. Food Res. 24:728.

CHAPTER III

VISUAL SELECTION OF HIGH AND LOW PIGMENT BEEF LOINS AS RELATED TO FROZEN DISPLAY COLOR UNIFORMITY BETWEEN LONGISSIMUS AND PSOAS MAJOR MUSCLES

Color variability between frozen beef muscles in skin-tight packaging presents marketing problems. This study was conducted to determine if selection of loins for low muscle heme pigment level would result in color uniformity between longissimus (L) and psoas major (PM) in frozen beef T-bone steaks.

Experimental Procedure

Thirty-six beef wholesale loins were selected to represent either a high or low pigment level on the basis of visual evaluation of muscles on both the rib and sirloin end.

Two replications were run on the display phase of this study. In the first replicate, ten loins were selected for each pigment level and in the second, eight were selected for each group. Loins represented USDA Good and Choice grades and were selected at a commercial packing plant. A 2.54 cm thick T-bone steak for display with psoas major (PM) large enough for color evaluation was cut on a power saw, scraped and trimmed. After 30 minutes of oxygenation, steaks were immediately placed in an NCG Ultra-Freeze Simulator Freezer and exposed to liquid nitrogen vapor in a step-wise temperature cycle beginning at -17.8 C and ending at -129 C. This crust freezing required about seven minutes. The crust frozen steaks were allowed to warm on the surface long enough to dissipate surface frost and

were immediately vacuum packaged in a skin tight package of DuPont Iolon film (O_2 permeability about 209 cc O_2/m^2/24 hrs/atm @ 22.2 C) using a DuPont MSP-1 packaging machine. They were immediately placed in a Hussman display case, -31.6 C at meat surface level, with defrost cycle two times per day, and 100 foot candles G.E. Delux Warm White fluorescent lighting (four, 40 watt tubes at 126 cm distance) at steak surface level, 24 hours a day for 35 days. Care was taken to insure exposure of the same muscle surface to the display lighting.

Subjective color scores, by two scorers, using the five point system as outlined in Kansas Circular #398, were taken after one and seven days of display.

Reflectance was scanned from 400 nm to 700 nm at a scan speed of 250 nm per minute using a Bausch and Lomb reflectance spectrophotometer. Reflectance at 630 nm was used, with a higher reflectance indicating a brighter red color.

After seven days of display, some steaks were selected as uniform or non-uniform steaks based on visual color score differences between longissimus (L) and PM muscles. Uniform steaks had 0.5 or less visual score units difference between these muscles and non-uniform had 1.5 or more difference.

The L and PM muscles used for analysis for heme pigment, ether extract, moisture, and protein were removed from the loin adjacent to the display steaks, allowed to oxygenate for 30 minutes, then packaged in Kraft-polyethylene laminated paper and stored in a Hussman freezer at -31.6 C until tests were conducted approximately two weeks later.

Samples were removed from the freezer, allowed to thaw sufficiently to be ground three times through a 1/8 inch plate, mixed and sealed in small polyethylene bags.

Total heme pigment was determined by the cyanomet method using $K_3Fe(CN)_6$ and KCN (Drabkin et al., 1935). The procedures for moisture involved 16 hours of drying in a forced air oven at 95 C; for ether extractables, the Soxhlet method; and for protein, Kjeldahl protein nitrogen x 6.25 (AOAC, 1970). Lipid oxidation was determined by the 2-thiobarbituric acid (TBA) method in accordance with Tarladgis et al., (1960).

Statistical analyses were done by analysis of variance (Snedecor and Cochran, 1971).

Results and Discussion

Characteristics of L and PM muscles from beef loins selected for high and low pigment are shown in Table 1. Total heme pigment demonstrated differences ($P<.01$) due to pigment level and muscle. Loin muscle pigment level was the presumed basis of visual selection, and this selection basis was successful in that low pigment loin muscles had lower ($P<.01$) total heme pigment levels than those selected for high pigment levels. Muscle heme pigment levels were as expected with higher pigment for PM muscle.

A difference ($P<.05$) due to pigment level selection was noted in percent ether extract. PM content of more ether extractables was in agreement with Schafer (1972). Higher pigmented muscles tended to accumulate or be associated with a higher level of ether extractables. Some previous studies (Beecher et al., 1965; Cassens, 1971) have found muscles of higher red fiber content such as the PM to contain more lipid. This was not found to be significant here, even though such a trend is evident. Percent protein demonstrated a difference ($P<.05$) due to muscle, with lower levels for PM. This agreed with Schafer (1972).

Moisture and TBA demonstrated no significant differences. However, TBA level tended to parallel the levels of both total heme pigment and ether extractables. Total heme pigment and ether extractables are major factors which may affect TBA level (Tarladgis et al., 1960). TBA values were determined on steak muscles from replicate II after 35 days of display. Muscles were highly discolored and mostly

TABLE 1. EFFECT OF SELECTING LOINS FOR HIGH AND LOW MUSCLE PIGMENT LEVEL ON LONGISSIMUS (L) AND PSOAS MAJOR (PM) CHARACTERISTICS

Variables	Low pigment		High pigment		F value significance[a]	
	L	PM	L	PM	Muscle	Pigment level
Total heme pigment (mg pigment/gm tissue)	3.97	4.66	4.58	4.98	**	**
Ether extract, %	5.08	5.96	6.61	7.61		*
Protein, %	21.84	21.55	21.62	21.03	*	
Moisture, %	72.45	71.82	70.65	70.98		
TBA[b] (mg malonaldehyde/ kg tissue)	0.91	0.97	0.93	1.11		

a = No pigment x muscle interaction ($P<.05$)

b = Measured on display steaks from replicate II

* = $P<.05$

** = $P<.01$

unsaleable at this time, but TBA values were not unduly high. This suggests that color deterioration is more rapid and occurs earlier than lipid oxidation.

Subjective color score means (Table 2) for frozen steak muscles (L and PM) after day one of display demonstrate differences due to muscle, pigment level, replicate, the interaction of replicate and muscle ($P<.01$) and scorer ($P<.05$). These results indicate that inherent muscle differences in muscle and pigment level affect meat color after freezing and skin-tight packaging. Lower scores (brighter color) for L agree with findings of Sandberg (1970), Santamaria (1970) and Fry (1972). Under conditions of oxygen limitation existant with skin-tight packaging the L, with lower pigment level, was brighter than PM which had higher pigment level.

Muscles from loins selected for low pigment, both L and PM, showed brighter color (lower scores) than those selected for high pigment levels. This agreed with the speculation of Schafer (1972) that pigment level was associated with color early in display. Presumably, darker color after one day display was due to increased myoglobin in reduced state as contrasted to oxygenated state and might have indicated a more severe oxygen limitation in the mini-environment surrounding muscles containing high pigment levels when packaged in a skin-tight package.

Reflectance at 630 nm (Table 2) for steak muscles (L and PM) from replicate II on day zero demonstrated differences due to selection for muscle, pigment level, and the interaction of muscle and pigment level

TABLE 2. EFFECT OF SELECTING LOINS FOR HIGH AND LOW MUSCLE PIGMENT LEVEL ON LONGISSIMUS (L) AND PSOAS MAJOR (PM) SUBJECTIVE COLOR SCORES[abc] AND PERCENT REFLECTANCE 630 NM

					F value significance					
	Low pigment		High pigment			Pigment				
Variables	L	PM	L	PM	Muscle (M)	level (PL)	Replicate (R)	RXM	MXPL	Scorer (S)
Visual score										
Day 1	1.4	2.6	1.7	2.9	**	**	**	**		*
Day 7	3.0	3.7	2.9	3.6	**			**		
Reflectance % @ 630 nm										
Day 0[e]	32.89	24.23	27.28	20.78	**	**			**	
Day 1[f]	30.97	21.22	31.40	21.65	*					
Day 7	26.63	17.33	26.61	19.53	**		**	**		
Uniformity[d]										
Uniform	7		5							
Indeterminate	8		11							
Non-uniform	3		2							

a = -36.1 C, 2 defrost cycles/day
b = 100 fc G.E. Delux Warm White, 24 hr/day
c = 1 (very bright red) to 5 (extremely dark)
d = Visual score difference between L and PM of 0.0 to 0.5 (uniform) .51 to 1.49 (indeterminate) 1.5 and greater (non-uniform) determined at day seven
e = Replicate I only
f = Replicate II only
* = P<.05
** = P<.01

($P<.01$). The higher reflectance of low pigmented L and PM muscles indicated a brighter red color and substantiated visual color score results for day one.

Percent reflectance of frozen steak muscles after day one of display demonstrated differences due to muscle ($P<.05$). Higher reflectance of L at 630 nm (brighter color) indicated that the lower pigmented of the two muscles (L and PM) was brighter after day one of display. This brighter color may be attributed to the possibility that less myoglobin is in the reduced state.

Subjective color score means for frozen steak muscles (L and PM) after seven days of display demonstrated differences due to muscle and interaction of replicate and muscle ($P<.01$). This tended to indicate that pigment level, replicate, and scorer differences that were significant on day one were no longer significant but may have had a slight influence on mean color scores at day seven. L muscles were still brighter (lower scores) than PM muscles. No significant differences were noted between low pigmented muscles (both L and PM) and those selected for high pigment levels. This would suggest that selection for pigment level was unsuccessful in obtaining T-bone steak muscles of brighter color after seven days of display, but had an effect on color early in display.

Percent reflectance at 630 nm for frozen steak muscles after seven days demonstrated differences due to muscle, replicate, and interaction of replicate and muscle ($P<.01$).

The difference in percent reflectance between L and PM muscles of low pigment group on day one and also of high pigment group was 9.75.

After seven days of display the differences were 9.3 units and 7.1 units respectively, but this interaction at day seven was not significant. Lower pigmented muscles tended to reduce more in percent reflectance than the higher pigmented muscles between day zero and day seven, but this difference was not statistically analyzed. Wang (1962) found that greater concentrations of myoglobin were more stable to oxidation.

The selection of beef loins in this study for high and low muscle pigment level apparently did not influence color uniformity between L and PM muscles after seven days frozen display.

REFERENCES

AOAC, 1970. "Official methods of Analysis". Assoc. Offic. Anal. Chemists, Washington, D.C.

Beecher, G. R., R. G. Cassens, W. G. Hoekstra, and E. J. Briskey. 1965. Red and white fiber content and associated post-mortem properties of seven porcine muscles. J. Food Sci. 30:969.

Cassens, R. G. 1971. Miscropic structure of animal tissues. The Science of Meat and Meat Products. W. H. Freeman and Co., San Francisco.

Drabkin, D. L. and J. H. Austin. 1935. Spectrophotometric studies. II. Preparation from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. J. Biol. Chem. 112:51.

Fry, J. A. 1972. Color stability of frozen beef steaks as affected by various commercial display lighting sources. M.S. Thesis. Kansas State University, Manhattan, Kansas.

Kansas State University. 1971. Beef color. Kansas Agric. Exp. Sta. Circ. 398.

Sandberg, M. L. 1970. 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. M.S. Thesis. Kansas State University, Manhattan, Kansas.

Santamaria, J. 1970. Effects of display light intensity on color stability of frozen beef cuts in transparent film. M.S. Thesis. Kansas State University, Manhattan, Kansas.

Schafer, D. E. 1972. Histochemical and biochemical characterization of bovine longissimus and psoas major muscles as related to frozen display color variations. Ph.D. Dissertation. Kansas State University. Manhattan, Kansas.

Snedecor, G. W. and W. G. Cochran. 1971. Statistical methods. The Iowa State Univ. Press, Ames, Iowa.

Tarladgis, B. G., B. M. Watts, M. T. Younathan, and L. R. Dugan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44.

Wang, J. H. 1962. Hemoglobin and myoglobin, in oxygenase. Hayaishi, O., Ed. Academic Press, New York.

CHAPTER IV

SUMMARY

Color variability between frozen beef muscles in skin-tight packaging presents marketing problems. This study was conducted to determine if selection of loins for low muscle heme pigment level would result in color uniformity between longissimus (L) and psoas major (PM) in frozen beef T-bone steaks.

Muscles were taken from ten loins each of high and low pigment level in the first replicate and from eight loins for each pigment level in the second replicate. Steaks were cut 2.54 cm thick, exposed to oxygen for at least 30 minutes, crust frozen with liquid nitrogen vapor in a step-wise temperature cycle beginning at -17.8 C and ending at -129 C with cycle time of about seven minutes, then skin-tight packaged using DuPont Iolon film in a DuPont MSP-1 packaging machine, and were displayed at -31.6 C with 100 fc G.E. Delux Warm White fluorescent lighting 24 hours a day for 35 days.

Visual color score (to nearest 0.5 point on 5 point visual scale) and percent reflectance at 630 nm were used to measure color after one and seven days display. Steaks were classified to be uniform (0.5 or less color score difference between L and PM), indeterminate or non-uniform (1.5 or greater color score difference between muscles) after seven days display. Total heme pigment (cyanomet method), percent ether extract, protein, and moixture were determined on samples removed adjacent to display steaks. TBA tests were conducted on steaks that had been displayed for 35 days.

Significant differences were found between L and PM muscles in almost every parameter studied. L contained a lower level of total heme pigment, higher protein level, and tended to have less ether extract. L was also brighter in visual color and showed a higher reflectance at 630 nm at zero time and after one and seven days of display. TBA values of steaks displayed 35 days tended to parallel total heme pigment and ether extract content, although no significant effects were found due to muscle or selection level.

Differences due to visual selection for pigment level were found for total heme pigment ($P<.01$) and ether extract ($P<.05$). L and PM muscles from loins selected for low pigment level had lower total heme pigment and less ether extract. Loins selected for low pigment level also had more desirable visual color after one day display and higher reflectance (brighter color) in replicate I steaks at zero time. Selection by pigment level had no significant effect on visual color score or percent reflectance after seven days of display. No difference was found between pigment level groups in number of uniform, indeterminate, or non-uniform colored steaks after seven days display.

Visual selection of high and low pigmented loins was successful in selecting steaks of uniform visual color after one day display and in selecting L and PM of high and low total heme pigment respectively but was not a good criterion of selection for steaks of color uniformity after seven days display.

APPENDICES

APPENDIX A

Visual Color Scores

1 = Very bright red

2 = Bright red

3 = Slightly dark red or brown

4 = Moderately dark red or brown

5 = Extremely dark red or brown

APPENDIX TABLE B. INDIVIDUAL VALUES OF REPLICATE I HIGH AND LOW PIGMENTED BEEF LOIN MUSCLES.

	Total heme pigment[a] mg/g		Ether extract[a] %		Protein[a] %		Moisture[a] %		Visual color scores Day 1		Visual color scores Day 7		Color reflectance % @ 630 nm Day 1		Color reflectance % @ 630 nm Day 7	
	L	PM	L	PM	L	PM	L	PM	L	PM	L	PM	L	PM	L	PM
High pigment																
2	4.51	5.93	5.90	4.34	21.83	20.76	71.456	74.430	2	3	2.25	3.5	36.5	18.9	38.3	27.2
4	4.69	4.75	4.50	6.05	21.82	22.75	72.069	70.791	2.25	3.5	3.5	4.5	27.0	13.5	29.9	25.3
6	4.49	5.35	6.99	6.33	22.57	22.39	70.598	71.232	2	3.25	2.5	3.25	19.4	14.9	29.3	18.5
8	3.82	4.95	4.88	5.18	22.35	21.19	72.163	72.568	1.5	3.25	3.5	4.5	36.0	17.0	45.7	26.1
10	4.41	5.49	6.24	6.10	22.08	21.41	69.607	70.965	1.5	3.5	3.75	4.5	31.2	18.0	43.4	18.8
12	4.64	5.44	2.51	8.26	22.35	21.31	74.070	69.401	2	3.25	3.25	4.5	22.5	20.0	32.7	18.4
14	4.17	5.00	3.32	5.17	21.45	20.47	73.821	73.741	1.25	3	1.75	3.75	21.0	21.0	34.0	23.2
16	4.70	4.95	3.57	5.75	20.87	21.08	73.946	72.385	2	3	3.25	4.25	32.7	22.6	42.0	16.4
18	5.03	4.07	9.16	21.57	20.76	18.85	71.14	59.44	2.5	3.5	2.25	4.25	20.4	33.5	30.8	28.5
20	5.35	5.25	8.45	8.06	20.11	21.67	69.990	69.960	2.25	3.75	4	5	26.1	28.4		
Low pigment																
1	4.20	5.32	5.87	5.73	22.57	22.33	71.406	71.910	1.5	2.5	3	3.75	36.2	22.0	50.8	29.9
3	3.60	4.47	6.50	4.94	21.42	21.93	71.383	72.131	1.5	3	2.5	4	39.0	28.5	43.8	24.8
5	3.27	4.22	6.75	6.68	21.16	21.93	71.663	70.641	1.5	3	2.5	4	35.0	19.4	41.0	25.8
7	4.38	5.04	8.29	9.61	21.14	20.14	69.564	70.297	1.75	3.25	3	3.5	31.8	20.5	39.7	17.6
9	4.50	4.72	4.58	4.93	21.59	20.16	71.671	73.479	1.5	3.25	3.5	4.25	29.0	23.9	36.2	18.0
11	2.82	5.48	3.88	3.43	22.02	21.70	71.656	72.767	1.25	3	3.25	3.75	33.7		49.5	27.0
13	5.05	4.74	3.66	4.81	21.92	21.56	73.170	72.439	1.25	3.25	3.25	4.25	34.7	22.4	39.2	16.7
15	4.76	4.66	6.79	4.73	20.69	21.07	71.539	73.421	1.25	3	2.75	4.25	31.8	20.6	37.7	23.0
17	3.80	4.43	4.60	4.08	21.89	22.17	72.743	73.507	1.5	3.25	4.	4.75	26.1	33.0	30.0	21.6
19	4.12	5.01	4.35	5.95	21.45	21.24	73.008	72.465	1.5	3.5	4	5	31.6	27.8		

APPENDIX TABLE C. INDIVIDUAL VALUES OF REPLICATE II HIGH AND LOW PIGMENTED BEEF LOIN MUSCLES

	Total heme pigment[a] mg/g		Ether extract[a] percent		Protein[a] percent		Moisture[a] percent		TBA ground		TBA displayed	
	L	PM	L	PM	L	PM	L	PM	L	PM	L	PM
High pigment												
1	4.71	5.07	7.31	11.11	21.92	21.38	70.068	66.600	1.63	1.69	.93	1.22
2	4.30	4.25	13.24	8.71	19.98	19.18	66.082	72.364	2.33	.46	.58	1.50
3	5.26	4.61	14.14	8.63	19.96	19.40	65.579	72.604	1.27	.42	1.83	.55
4	4.48	4.72	5.62	7.73	21.77	21.74	72.450	69.586	1.54	1.15	.42	.54
5	4.43	5.27	8.73	5.77	22.07	22.12	68.937	71.366	2.09	1.33	.52	2.15
6	4.22	4.58	5.71	7.02	22.26	20.71	71.449	72.262	1.76	1.48	1.17	.79
7	4.29	4.87	6.01	7.01	22.59	20.17	71.522	72.714	2.40	.91	1.04	1.00
8	4.88	5.04	2.64	4.11	22.50	22.06	75.330	74.183	.68	.73	.98	1.11
Low pigment												
1	3.48	4.35	3.11	7.41	22.57	22.55	73.872	69.525	1.79	1.02	1.49	.80
2	4.34	4.83	5.75	7.80	22.44	21.21	71.803	71.154	1.74	1.15	1.23	.90
3	3.55	4.85	5.29	7.07	21.83	21.96	72.285	71.382	.70	.64	.34	.92
4	3.30	4.29	4.45	6.53	22.79	22.19	72.349	70.989	.92	.33	.50	1.12
5	3.81	3.81	3.80	4.74	22.24	22.14	73.431	72.723	1.34	.57	1.20	1.08
6	4.17	4.26	4.10	7.13	22.67	21.44	73.777	71.632	1.92	1.31	.94	1.06
7	4.02	4.88	4.81	6.72	20.83	19.54	73.221	72.864	.66	.88	.74	.83
8	4.41	4.57	4.86	5.08	21.84	22.64	74.242	70.397	.78	.60	.84	1.05

a = Fresh weight basis

APPENDIX TABLE C (cont.). INDIVIDUAL VALUES OF REPLICATE II HIGH AND LOW PIGMENTED BEEF LOIN MUSCLES

	Visual color scores				Color reflectance at 630 nm			
	Day 1		Day 7		Day 1		Day 7	
	L	PM	L	PM	L	PM	L	PM
High pigment								
1	2	3	3.5	4.5	28.8	13.1	21.0	10.8
2	2.25	2.25	3.75	3	33.5	30.5	18.2	27.2
3	1.75	2.5	3.5	3.25	37.0	22.7	22.9	20.3
4	2	2.5	3.75	3.75	27.8	21.0	25.0	17.9
5	1.25	2.75	3.25	4.25	31.2	23.0	25.7	23.3
6	1.25	2.75	3.5	3.5	25.9	26.8	19.6	27.8
7	2	3.25	3.75	3.75	32.0	15.4	17.9	15.8
8	2.25	2.5	3.5	3.75	35.0	20.7	22.5	22.3
Low pigment								
1	2	2.5	3.75	3.75	30.5	20.5	19.2	9.9
2	2	2	3.75	3.25	28.9	31.8	20.2	20.0
3	1.25	2.2	3.5	3.75	35.5	19.5	24.1	19.4
4	1.5	2.5	3.5	4	27.0	18.5	24.8	20.0
5	1.5	2.75	3.25	4.25	33.5	21.0	27.0	15.8
6	2	2.5	4	4.5	25.0	16.0	16.1	18.0
7	1.5	2.5	3.25	4.5	38.0	18.4	30.3	13.1
8	1.5	3	3.5	4.5	29.4	24.1	23.5	13.7

APPENDIX D. DETERMINATION OF TOTAL HEME PIGMENT IN MEAT

Procedure:

1. Weigh 10 g of sample (ground, mixed 3 times).

2. Blend with 20 ml of .006 m acetate buffer (pH 4.5) in a Virtis "45" blender on medium speed for 1 min.

3. Centrifuge in IEC refrigerated centrifuge model B-20 at 15,000 g for 10 min at 11 C. Low temperature will solidify the fat from the sample and make it easier to remove before filtering.

4. Filter supernatant once through Whatman #2 paper. Wash paper with small aliquots of buffer.

5. Re-suspend the precipitate in 20 ml buffer, centrifuge, filter, and combine the filtrate from step 4 into a 100 ml volumetric flask. Make to volume with buffer.

*6. Place .4 ml of 16.2 mM $K_3Fe(CN)_6$ and .4 ml of 21.6 mM KCN in a test tube. Then add 10 ml of filtrate, insuring the filtrate is added down side of tube to prevent turbid solution. Wait 2 minutes before mixing to prevent turbidity.

7. Read absorbance at 540 nm in a colorimeter or a spectrophotometer.

8. Zero the above machine using distilled H_2O. Read blank which contains everything except sample. Subtract this reading from the absorbance readings of samples.

$$\text{mg of pigment/g of tissue} = A^{o} \times 183{,}600$$

A^{o} = absorbance at 540 nm

$$183{,}600 = \frac{17{,}000 \times .108 \times 1000}{\text{gm of sample}}$$

*Insure both the $K_3Fe(CN)_6$ and the KCN are prepared fresh each day and held under refrigeration when not in use. By doing this, the precision between duplicates is increased.

VISUAL SELECTION OF HIGH AND LOW PIGMENT BEEF LOINS AS RELATED TO FROZEN DISPLAY COLOR UNIFORMITY BETWEEN LONGISSIMUS AND PSOAS MAJOR MUSCLES

by

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AN ABSTRACT OF A MASTER'S THESIS

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Color variability between frozen beef muscles in skin-tight packaging presents marketing problems. This study was conducted to determine if selection of loins for low muscle heme pigment level would result in color uniformity between longissimus (L) and psoas major (PM) in frozen beef T-bone steaks.

Muscles were taken from ten loins each of high and low pigment level in the first replicate and from eight loins for each pigment level in the second replicate. Steaks were cut 2.54 cm thick, exposed to oxygen for at least 30 minutes, crust frozen with liquid nitrogen vapor in a step-wise temperature cycle beginning at -17.8 C and ending at -129 C with cycle time of about seven minutes, then skin-tight packaged using DuPont Iolon film in a DuPont MSP-1 packaging machine, and were displayed at -31.6 C with 100 fc G.E. Delux Warm White fluorescent lighting 24 hours a day for 35 days.

Visual color score (to nearest 0.5 point on 5 point visual scale) and percent reflectance at 630 nm were used to measure color after one and seven days display. Steaks were classified to be uniform (0.5 or less color score difference between L and PM), indeterminate or non-uniform (1.5 or greater color score difference between muscles) after seven days display. Total heme pigment (cyanomet method), percent ether extract, protein, and moisture were determined on samples removed adjacent to display steaks. TBA tests were conducted on steaks that had been displayed for 35 days.

Significant differences were found between L and PM muscles, selected for high and low pigment levels, in almost every parameter

studied. L contained a lower level of total heme pigment, higher protein level, and tended to have less ether extract. L was also brighter in visual color and showed a higher reflectance at 630 nm at zero time and after one and seven days of display. TBA values of steaks displayed 35 days tended to parallel total heme pigment and ether extract content, although no significant effects were found due to muscle or selection level.

Differences due to visual selection for pigment level were found for total heme pigment ($P<.01$) and ether extract ($P<.05$). L and PM muscles from loins selected for low pigment level had lower total heme pigment and less ether extract. Loins selected for low pigment level also had more desirable visual color after one day display and higher reflectance (brighter color) in replicate I steaks at zero time. Selection by pigment level had no significant effect on visual color score or percent reflectance after seven days of display. No difference was found between pigment level groups in number of uniform, indeterminate, or non-uniform colored steaks after seven days display.

Visual selection of high and low pigmented loins was successful in selecting steaks of uniform visual color after one day display and in selecting L and PM of high and low total heme pigment respectively, but was not a good criterion of selection for steaks of color uniformity after seven days display.