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Myoglobin Content and Color of Raw Pork Loin Roasts as Affected by Freezing at Two Rates

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I am submitting herewith a thesis written by Jane Shellabarger Nocito entitled "Myoglobin Content and Color of Raw Pork Loin Roasts as Affected by Freezing at Two Rates." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Bernadine Meyer, Major Professor

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Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 20, 1972

To the Graduate Council:

I am submitting herewith a thesis written by Jane Shellabarger Nocito entitled "Myoglobin Content and Color of Raw Pork Loin Roasts as Affected by Freezing at Two Rates." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

Bernadine H. Meyer
Major Professor

We have read this thesis
and recommend its acceptance:

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Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

MYOGLOBIN CONTENT AND COLOR OF RAW PORK LOIN ROASTS AS
AFFECTED BY FREEZING AT TWO RATES

A Thesis

Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Jane Shellabarger Nocito
December 1972

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ABSTRACT

Longissimus dorsi and psoas major muscles from fresh and frozen paired pork loin roasts were evaluated for myoglobin content and color reflectance. One of each paired roast was analyzed fresh and the other analyzed after freezing and thawing. Myoglobin in the thaw drip from frozen roasts was measured, also. Two freezing rates were tested: fast freezing in an air blast freezer at -27° to -29°C and slow freezing in a home freezer at -18°C . Roasts were stored for 28 days before thawing.

Myoglobin in the longissimus dorsi was significantly ($P < 0.001$) lower than in psoas major muscle. Less myoglobin ($P < 0.05$) was found in fresh than frozen thawed roasts; but there was no difference ($P > 0.05$) in myoglobin content associated with freezing rate. Apparently, myoglobin extraction from frozen muscles was more efficient than from fresh muscles. No difference was detected in the total myoglobin in the thaw drip of blast and home frozen roasts.

Significant ($P < 0.001$) differences between muscles were detected for lightness index and purity. No significant ($P > 0.05$) differences were found between dominant wavelength, purity, or lightness index and freezing treatment. For fresh muscles, purity and lightness index were related to myoglobin content.

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CHAPTER I

INTRODUCTION

Centralized freezing and prepackaging of retail cuts offers an innovation to lower meat processing costs and to meet the forecasted demand for meat. From 1970 to 1975, sales of frozen meats are projected to rise 71.6 percent (Anonymous, 1972). In a survey to determine consumer reactions to agricultural products, 87 percent of the 3,099 homemakers froze fresh meat at home; but few were interested in purchasing previously frozen raw meat (Anonymous, 1969). Consumer resistance to prefrozen meat has been due to the appearance of meat which has lost its color and attractiveness and to a lack of standards for judging frozen meats. One of the salient criteria by which the consumer judges meat quality is the color of the lean. Thus, research is needed in the area of frozen meat color.

The color of meat is a result of the chemical state of the myoglobin heme group, the concentration of myoglobin, as well as the fat deposition and the surface characteristics. Myoglobin is a complex muscle pigment primarily responsible for the red color of meat. The respiratory pigment is composed of an apoprotein, the globin, and the prosthetic group, a heme molecule which is an iron porphyrin compound.

Many conflicting opinions concerning freezer damage to meat quality have been reported (Aref, 1965). One theory proposed that slow freezing of meat results in greater cell rupture, increased drip and loss of more water-soluble material than freezing at faster rates.

Since myoglobin is water-soluble, the pigment is partially lost in the exudate of frozen, thawed meats. Limited research has been devoted to relating color reflectance measurements and myoglobin content of frozen pork muscle, which is the major interest in the present investigation.

The purpose of the present investigation was to determine the effect of freezing rate on myoglobin content of pork loin roasts and thaw drip and on color reflectance of roasts. Fresh and frozen, thawed paired pork loin roasts, containing longissimus dorsi and psoas major muscles, were compared chemically for myoglobin and physically for color reflectance. Roasts were frozen at one of two rates: in an air blast freezer at -27° to -29°C or in a home freezer at -18°C .

CHAPTER II

REVIEW OF LITERATURE

I. MEAT PIGMENTS

The color of meat is attributable to several components. The cytochromes are red heme pigments which contain iron in porphyrin protein complexes (Bodwell and McClaim, 1971). Vitamin B₁₂ contains a porphyrin ring similar to the cytochromes but also contains a cobalt atom instead of an iron. The flavins, yellow coenzymes, are involved with cytochromes in electron transport in the cells. These pigments are present in meat in such small quantities that they contribute little to total meat color. Hemoglobin, the blood pigment, may comprise 20 percent of the pigment present in a well-bled animal (Clydesdale and Francis, 1971). A hemoglobin molecule contains four heme groups.

The primary pigment of the lean portion of meat is myoglobin, a complex muscle protein (Solberg, 1970). The pigment is composed of an apoprotein, the globin, and the prosthetic group, a heme molecule which is an iron porphyrin compound. The porphyrin ring consists of four pyrroles linked together by methene bridges. The side chains attached to the porphyrin portion of the molecule are methyl, vinyl, and propyl groups. Iron is covalently bonded to nitrogen of the pyrrole groups.

Function of Myoglobin

Oxygen from the lungs is transported by hemoglobin in the blood into the capillaries. The oxygen then diffuses into the muscle tissues

where it is bound by myoglobin for future use in metabolic activity of the animal. The sixth coordination position of myoglobin provides for the respiratory function of the molecule in that the site is available for complexing of any atom which has an electron pair to donate (Solberg, 1970). Thus, myoglobin is essentially a storage mechanism for oxygen in the cells and reflects its role in the quantities of pigment found in the tissues.

Chemical State of Myoglobin and Color

The color of meat is affected by the chemical state of the heme pigment (Solberg, 1970). Complexes of heme with ligands are of two types: the iron of the heme may be in the ferrous or ferric state. Myoglobin with an iron atom in the reduced state (Fe^{++}) provides a purplish red color evident in the interior of fresh raw meat. Oxymyoglobin, which also has a reduced iron atom, contains a covalently bonded oxygen molecule. The oxygenated form produces a bright red color in meat. Oxidation of the iron in myoglobin or oxymyoglobin to the ferric state (Fe^{+++}) yields metmyoglobin which is brown in color. On the surface of fresh meat, the color cycle is a reversible dynamic conversion of metmyoglobin to myoglobin and oxymyoglobin, due to the presence of reducing substances and oxygen.

Muscle Concentration of Myoglobin

The quantity of myoglobin is affected by species, age, extent and type of muscular activity, and oxygen availability of the animal (Lawrie, 1950 and Bodwell and McClain, 1971). Research workers do not agree as to the average myoglobin content of beef. The average myoglobin

concentration of beef muscle of 2.43 mg per g of wet tissue reported by Fleming et al. (1960) and 2.80 mg per g of wet tissue reported by Rickansrud and Hendrickson (1967) were somewhat lower than the range of 4-10 mg per g of wet tissue given by Clydesdale and Francis (1971). The latter group reported veal and pork to have an average myoglobin content of 1-3 mg per g of wet tissue.

Myoglobin concentration was found to increase proportionally with age of swine (Lawrie, 1950). The rate of increase was rapid from birth to one year. After one year, the longissimus dorsi was the only muscle which continued to show significant increases.

Myoglobin content varies with the particular muscle within a species. In four beef muscles studied by Rickansrud and Hendrickson (1967), myoglobin concentration was highest in the biceps femoris, 3.64; followed by the longissimus dorsi, 3.18; psoas major, 2.40; and lowest in the semitendinosus, 1.99 mg per g of wet tissue. In four pork muscles, on a dry weight basis, the percent of myoglobin was highest in the psoas major, 1.60; followed by the diaphragm, 1.35; the longissimus dorsi, 0.85; and lowest in the heart, 0.79 (Lawrie, 1950). Muscle activity affects myoglobin content. Pigs confined to pens just adequate to accomodate them had a lower myoglobin content in the longissimus dorsi than active pigs of the same age (Lawrie, 1950).

Oxygen availability to the muscle is influenced by the amount of myoglobin present. The wing muscles of birds are lower in myoglobin than the rest of the sketal muscles because of a highly efficient wing blood supply which satisfies oxygen demand (Clydesdale and Francis, 1971). The ability of whales to remain submerged for periods as long as

an hour without breathing is accounted for by the animal having the highest amount of myoglobin of all mammals.

Relationship of Myoglobin Content and Color

Meat color is dependent upon the myoglobin concentration in the muscle. Myoglobin content and fiber type are positively correlated (Briskey and Kauffman, 1971). Red fibers have a high myoglobin content and white, a low. Varying gradations of intermediate fibers are usually present. In porcine muscles, red fibers are found in discrete groups or clumps of about five to seven fibers toward the center of the bundle and are surrounded by white fibers (Moody and Cassens, 1968). In redder muscles, such as the psoas major, almost the entire center of the bundle is composed of one large group of red fibers surrounded by one or two layers of white fibers at the periphery. Beecher et al. (1965) listed serratus ventralis and the interior of the biceps femoris and the semitendinosus of the porcine carcass as red muscle. The longissimus dorsi, gluteus medius, and exterior of the biceps femoris and semitendinosus were classified as white muscle.

Measurement of Myoglobin

Myoglobin was first crystallized by Theorell (1932). Finely ground horse heart meat was extracted with water, and foreign protein was precipitated from the extract with lead acetate. The filtrate was then repeatedly dialyzed against solutions of saturated ammonium sulfate until characteristic fan-shaped clusters of needle-like crystals appeared. Prior to Theorell's isolation of myoglobin, a distinct differentiation between hemoglobin and myoglobin was not generally accepted. Morgan

(1936) observed that while myoglobin appeared to be extremely soluble in a 3 M phosphate solution at a pH of 6.6, hemoglobin was not soluble under these conditions. Thus, a simple method was provided for the separation of hemoglobin and myoglobin.

After the basic properties of myoglobin were reported, workers developed several methods to quantitatively estimate myoglobin in meat extracts. Shenk et al. (1934) were the first to approximate the concentration of hemoglobin and myoglobin in extracts containing the oxygenated pigments. In 1935, Drabkin and Austin determined total pigment from washed blood cells by measuring absorbance of cyanmethemoglobin at 540 nm. Hemoglobin was converted to the cyanmet- derivative by the addition of potassium ferricyanide (final concentration of 0.6 mM per liter) and potassium cyanide (final concentration of 0.8 mM per liter to the solution). An extinction coefficient of 11,500 at 540 nm for cyanmethemoglobin was used to convert optical density readings to mM per liter. In several studies an extinction coefficient of 11,300 at a wavelength of 540 nm was used for measuring cyanmetmyoglobin in extracts of rat, human, horse, dog, and beef muscle (Crandall and Drabkin, 1946 and Drabkin, 1950). Ginger et al. (1954) determined myoglobin as the cyanmet- compound in pork muscles but used an extinction coefficient of 11,500 at a wavelength of 540 nm.

In another approach, the concentration of myoglobin and hemoglobin was measured after conversion to carbon monoxide derivatives (DeDuve, 1948 and Poel, 1949).

Fleming et al. (1960) evaluated procedures for estimating chromoproteins for accuracy and adaptability of analytical technique. The

methods studied were those of cyanmet- (Ginger et al., 1954 and Drabkin, 1950), carbonyl- (DeDuve, 1948 and Poel, 1949) and oxy- (Shenk et al., 1934) myoglobin derivatives. The cyanmet- and oxymyoglobin procedures yielded values which were very close but lower than the values obtained by the carbonylmyoglobin method. Though the carbonyl- procedure was suggested for future use, values obtained for all three methods were noted as relative.

Using quantitative extraction methods, the concentration of myoglobin and its derivatives in fresh or frozen red meats frequently does not yield results that are closely related to color as seen by the human eye (Doty, 1960). This is explained by the fact that cyanmetmyoglobin measurement of meat samples does not differentiate between the chemical states of the pigment, the distribution of the pigments, or the state of hydration of the meat surface. These factors all affect subjective color evaluation of meat.

II. MEAT COLOR

The color of a medium as perceived by the human eye is due to the selective absorption of a portion of the white light of the sun or of artificial light by molecules or atoms (Mackinney and Little, 1962). For example, a substance will appear green if it reflects or transmits the wavelengths in the green portion of the visible spectrum and absorbs adequate visible radiations in the red and yellow regions.

Objective testing of color is a measurement of the physical nature of the light reflected or transmitted from the substance being viewed. The human eye is capable of perceiving radiations in the visible spectrum

from 380 to 760 nm.

Sample preparation and presentation affect color readings obtained by an instrumental method. Reflectance is a surface property of opaque objects such as meat. Thus, the surface characteristics of the sample such as texture, moisture, particle size, shape and orientation, granularity and degree of packing are important variables which must be carefully controlled (Mackinney and Little, 1962). Optimum results are obtained with flat, opaque, uniformly colored surfaces. These characteristics are seldom found in foods. Usually, solid foods are presented to the sample port of an instrument unaltered or slightly altered by flattening, homogenizing, grinding, or controlling particle size. The degree of color uniformity in the various parts of the sample also influences readings. With meat, for instance, reflectance increases with an increase in intramuscular fat (van der Oord, 1971). Any substance, glass, lucite, or other materials, interposed between the sample and the sample port is a factor which affects the final reflectance readings. Also, exposure of the surface to light or air, initial sample temperature, and heat from the instrument may influence color.

In addition to intramuscular fat distribution, other inherent characteristics of meat such as water-holding capacity, moisture content, pH, total protein content, total pigments and myoglobin content influence color measurements. Using the reflectance attachment of a Zeiss VSU-1 spectrophotometer, Janicki et al. (1967) found the only factor influencing the dominant wavelength or hue of minced raw fresh pork was pH. Nonsignificant correlations were found between hue and properties such as total pigments, water-holding capacity, moisture content, and total

protein content. Color lightness decreased with increased pigment content, water-holding capacity, and pH. With raw frozen, thawed pork, Akin (1971) reported reflectance values of longissimus dorsi muscles as measured by the Kollmorgen Color Eye Colorimeter. Color differences between samples were associated with chemically measured myoglobin ($r = 0.791$, $P < 0.01$); color lightness decreased with increased pigment content ($r = -0.631$, $P < 0.01$).

III. FREEZING AND THAWING OF MEAT

Freezing of meat has been known as an excellent method of preservation for the maintenance of fresh color, flavor, and palatability. The meat packaging industry employs temperatures of approximately -29° to -40°C for freezing meat in air blast tunnels or rooms (Bratzler, 1968). (All temperatures in the Review of Literature will be cited as reported in the literature.) The freezing rate of commercially processed meat is influenced not only by temperature but also by air movement, packaging material, the amount and location of muscle fat, and moisture content.

When meat is exposed to freezing temperatures, its own temperature drops gradually until it reaches the range at which the unbound water of the material begins to change into ice (Aref, 1965). During ice formation, the temperature of the meat remains on a plateau until almost all the unbound water turns into ice. A gradual drop in temperature is usually observed as the ice causes a constant increase in the concentration of the remaining solution and therefore, lowers the freezing point. Approximately 82 percent of the water in meat is frozen at

-5°C (Borgstrom, 1968). At lower temperatures, more water is frozen but in amounts disproportionate to the temperature (Aref, 1965). The freezing point continues to drop until all unbound water is changed to ice. Then the temperature of the meat gradually decreases until it reaches the temperature of the freezing environment.

Effect of Freezing Rate

Freezing rate influences not only the location of ice formation but also protein denaturation. Quick freezing produces tiny ice crystals within the cells leaving the meat unchanged structurally (Aref, 1965). At slow freezing rates, large extracellular ice crystals are formed. As freezing progresses, the remaining extracellular fluid increases in ionic strength and by osmotic pressure is able to draw water from the cell interiors of the muscle (Cassens, 1971). The structure is distorted by large ice crystals and protein denaturation occurs because of the high ionic strength of the extracellular fluid.

Koonz and Ramsbottom (1939) and Hiner and Hankins (1947) reported specific temperatures and location of ice formation. Slow freezing at -17°C produced intercellular crystals. The large ice formations tended to compress the fibers and connective tissue. Freezing at -40°C produced intrafiber ice crystals. Very fast freezing at -150°C resulted in evenly distributed ice crystals that were inconspicuous in size. The fastest rate yielded fewer and smaller cellular distortions.

The relative amount of damage to cells of a sample medium subjected to various rates of freezing is not of universal agreement (Cassens, 1971). When compared to a slow freezing rate, a fast rate of

freezing was noted as resulting in less cell damage and a minimum of drip containing less water-soluble constituents (Borgstrom, 1968). There are three possibilities for damage caused by freezing meat (Cassens, 1971). Cell damage may be due to ice crystal formation, increased osmotic pressure, and denaturation of the colloidal cell constituents. Although some workers assumed that the ice crystals produced by slow freezing methods tore the cells apart, other researchers noted that intercellular crystals, by themselves, were not necessarily damaging. Few large intercellular crystals were proposed as being less destructive than denaturation produced by the formation of many small intracellular crystals. Recently, cell damage has been analyzed by the measurement of the relative quantities of cellular components released into the expressible intercellular fluid.

Thaw Drip

Freezing and thawing of meat usually causes loss of water-holding capacity as evidenced by the leakage of fluid from meat during and after thawing (Paul, 1972). Increased protein denaturation and ionic concentration as well as high fat level cause a decrease in water-holding capacity.

The effects of freezing rate and ice crystal size on thaw drip were studied by Ramsbottom and Koonz (1941). Upon thawing at 50°F, beef steaks which were frozen at 10°F had 52 percent greater loss in drip than steaks which were frozen at -30°F.

The exudate contains soluble nutrients, sugars, amino acids and certain proteins, including myoglobin which is a water-soluble

sarcoplasmic protein. The amount of such constituents is directly related to the amount of thaw drip. A substantial amount of red exudate from frozen, thawed pork roasts was noted by Akin (1971). Thus, lower myoglobin content in frozen, thawed as compared with fresh roasts may be partially attributed to translocation of myoglobin into the drip.

Color of Frozen Meat

Investigators agree that slowly frozen meat appears darker than fresh meat (Ramsbottom and Koonz, 1941). Several reasons account for the darker color. In slowly frozen meat, large ice crystals and surface oxidation contributed to the dark color. Upon thawing, only metmyoglobin and methemoglobin were responsible for the permanent discoloration. However, at 1°C the concentration of metmyoglobin in whole beef muscles remained virtually constant from five to twenty-eight days of storage (Ledward, 1970).

Fast frozen meats appear lighter than fresh because of the reflectance of the light from the well-distributed small ice crystals. Tuma (1971) found freezing at -70°F or lower for a short time produced a brighter, more desirable color than steaks subjected to higher freezing temperatures for a longer time.

Limited research has been devoted to the comparison of the color of fresh and frozen meat as analyzed by reflectance. Lentz (1971) assessed the color of beef steaks immediately before and after freezing by comparison with Munsell color chips. Generally, the prefreezing color of most of the samples was 7.5 R 4/8 although a few were lighter, 7.5 R 5/8. Color change caused by freezing was small and tended to make

samples more reddish, 7.5 R 4/10. Under the conditions of the experiment, air blast (-20°F) freezing yielded color similar to that of unfrozen beef. Studies comparing color reflectance of fresh and frozen, thawed pork were not found in the literature.

CHAPTER III

PROCEDURE

I. PLAN OF STUDY AND SOURCE OF MEAT

Myoglobin content and CIE color values were determined on longissimus dorsi and psoas major muscles of 16 paired pork loin roasts, one of which was analyzed fresh and the other analyzed after freezing and thawing. Eight pairs of pork loins, 14 to 16 lb each, were selected 72 hours post-mortem from carcasses in the processing line at a local packing plant. The loins with an average color score of 2.5 were subjectively chosen using a 1 to 5 color classification system developed by Iowa State University (1969). In this classification, a score of 2 is described as slightly pale, soft, and watery; and 3 is described as normal grayish pink in color. Two paired sections, three inches in length, were removed from the lumbar region of the loins to provide roasts, weighing 1-1/4 to 2 lb, containing the two muscles of different pigment contents and colors. Two rates of freezing were used: rapid freezing in an air blast freezer at -27° to -29°C and slow freezing in still air in a home freezer at -18°C. (All temperatures reported in this study are in the Centigrade scale.) Roasts from each carcass were randomly assigned to the freezing treatments.

Analysis of the unfrozen roasts was begun one hour after the loins were procured. The longissimus dorsi and psoas major muscles were excised from the roast, and external fat and connective tissues removed.

The muscles were ground separately using a perforated plate with openings 10 mm in diameter for the first grinding and 4 mm in diameter for the second and third grindings. The ground meat with any exuding juice was collected in a plastic bag. The samples were mixed well before testing.

The other roast of each pair was frozen to -17°C at one of two rates and stored for 28 days. To approximate usual practices, roasts frozen in the blast freezer were wrapped in moisture-vapor-proof paper after freezing and roasts frozen in the home freezer were wrapped before freezing. The rate of freezing the roasts in the blast and home freezer was recorded by a multipoint temperature recorder. After freezer storage, the roasts were weighed and thawed in the refrigerator at 2°C for 48 hours in a pan covered with plastic wrap to prevent moisture loss. The weights of the thawed roasts and the thaw drip were recorded separately. The muscles were removed and ground as explained for the fresh roasts. The thaw drip was retained for measurement of myoglobin content.

II. METHODS OF EVALUATION

Myoglobin Content

Myoglobin was determined quantitatively according to the procedure of Ginger et al. (1954) with minor modifications.

Twelve grams of the ground longissimus dorsi and psoas major were extracted three times with distilled, demineralized water as follows: 4 hours with 9 ml of water; 2 hours with 5 ml of water; and overnight with 5 ml of water. Each duplicate sample was stirred periodically during the first and second extractions. After each extraction, samples were centrifuged for 15 minutes at $4,600 \times G$ at 6°C . The

supernatants from the three extractions were combined in a graduated centrifuge tube.

The meat extracts were adjusted to a pH of 7.0 with a 0.5 M phosphate buffer, pH 8.3. Saturated basic lead acetate equal to one-fourth the volume of the combined supernatants and buffer was added. Precipitated foreign proteins were removed by centrifuging at 2,000 x G at room temperature and filtering through Whatman No. 2 filter paper. Before filtering, the volume of the extract was recorded.

To a 10 ml aliquot of the filtrate, 2.04 g mono- and 2.61 g of dibasic potassium phosphate were added to adjust the pH of the extract to 6.6 and to bring the total phosphate concentration to 3 M which precipitated hemoglobin and other foreign proteins. The precipitate was removed by centrifuging at 2,000 x G for 15 minutes at room temperature followed by filtration with Whatman No. 42 filter paper.

A 5 ml aliquot from the resulting filtrate was converted to cyanmetmyoglobin derivative by addition of 0.03 ml of 0.1 M potassium ferricyanide and 0.04 ml of 0.1 M sodium cyanide. The absorbance was measured in a Bausch and Lomb Spectronic 20 at 540 nm.

The weighed drip of the thawed roasts was adjusted to a pH of 7.0 with a phosphate buffer of pH 8.3. The same procedure as described for the meat extracts was then followed for myoglobin determinations.

The equation used for calculating mg of myoglobin per g of wet tissue or drip from absorbance readings was:

$$\frac{\text{OD} \times \text{Vol of extract (liters)}}{\frac{\text{Extinction coefficient of Mb}}{\text{Length of light path}}} \times \frac{1000 \text{ mg/g} \times \text{Mol wt of Mb}}{\text{Wt of meat sample (g)}}$$

The molecular weight of myoglobin was assumed to be 17,000 g per mole (Kendrew, 1963). The extinction coefficient for cyanmetmyoglobin was based on Drabkin's (1950) value of 11,300. Since the Bausch and Lomb Spectronic 20 has a light path length of 1.17 cm, the extinction coefficient in the formula was adjusted accordingly (Ewing, 1960).

Color Reflectance

The Kollmorgen Color Eye Colorimeter readings of X, Y, Z, and X' were taken on duplicate samples of ground longissimus dorsi and psoas major muscles. The samples were wrapped in oxygen-permeable and moisture-proof wrap, Goodyear Vita Film, to prevent moisture loss. The samples were held in the refrigerator at 2°C for 1-1/2 hours to expose the surfaces to oxygen.

The readings from the Vita Film wrapped samples measured percent reflectance at 610 nm (X), 550 nm (Y), 445 nm (Z), and 435 nm (X') in relation to a white vitrolite standard. The readings were converted to CIE chromaticity coordinates x and y and to a lightness index. The x and y values were plotted on a CIE chromaticity diagram to determine dominant wavelength and purity of the color of the pork samples. The equations for the conversion of Color Eye readings to CIE values may be found in the Appendix (page 53).

Moisture and Fat Determination

Moisture content and ether extract were determined on duplicate samples from each fresh, blast frozen, and home frozen longissimus dorsi and psoas major muscles. The average percent fat-free-dry weights were calculated to account for moisture and fat variations between fresh

and frozen, thawed meat samples.

Approximately 5 g meat samples were weighed by difference to the nearest mg into Whatman 22 x 8 mm single thickness extraction thimbles. After drying overnight in a vacuum oven at 65°C, the samples were weighed. Fat was then extracted with petroleum ether for 6 hours in a Goldfish apparatus. After evaporating the ether, samples were redried for a 2 hour period and reweighed. Average percent fat-free-dry weight was calculated.

Analysis of Data

The myoglobin content of fat-free-dry tissue of fresh, blast frozen, and home frozen muscles was evaluated utilizing a three-way analysis of variance to study the variation that existed between two muscles, three treatments, and eight carcasses. The appropriate least squares procedure was used to analyze disproportionate and unequal subclasses (Harvey, 1960). When a significant F value for a factor was obtained, the means were partitioned by the Duncan's New Multiple Range Test (Kramer, 1956).

Percent of myoglobin transferred to the thaw drip was calculated. To compare the effect of freezing rate on the percentage of myoglobin transferred to the thaw drip, a paired Student's "t" test was applied (Steele and Torrie, 1960).

The myoglobin content of each fresh muscle on a fat-free-dry basis was calculated using the following equation:

$$\text{Mb in fresh muscle (mg)} = \frac{\text{Wt of fresh muscle (g)} \times \frac{\text{mg Mb}}{\text{g fresh muscle}}}{\% \text{ Fat-free-dry wt of fresh muscle}}$$

The theoretical myoglobin content of each frozen muscle on a fat-free-dry basis was calculated using the following equation:

$$\text{Mb of frozen muscle (mg)} = \frac{\text{Wt of fresh pair mate muscle (g)} \times \frac{\text{mg Mb}}{\text{g frozen muscle}}}{\% \text{ Fat-free-dry wt of frozen muscle}}$$

The total myoglobin lost in the thaw drip was calculated using the following equation:

$$\text{Mb in drip (mg)} = \text{Wt of drip} \times \frac{\text{mg Mb}}{\text{g drip}}$$

The theoretical percentage of myoglobin of each frozen roast compared to the myoglobin content of the paired fresh roast was calculated according to the following equation:

$$\text{Percent Mb recovered} = \frac{\text{Mb in frozen LD (mg)} + \text{Mb in frozen PM (mg)} + \text{Mb in drip (mg)}}{\text{Mb in fresh LD (mg)} + \text{Mb in fresh PM (mg)}} \times 100$$

The CIE values of fresh, blast frozen, and home frozen muscles were evaluated utilizing a three-way analysis of variance to study the variation that existed among two muscles, three treatments and eight carcasses. The appropriate least squares procedure was used to analyze disproportionate subclasses (Harvey, 1960). When a significant F value was obtained, the means were partitioned by the Duncan's New Multiple Range Test (LaClerq, 1970).

For the longissimus dorsi and for the psoas major muscles, simple correlation coefficients were computed between CIE values and myoglobin content of the fresh, blast frozen, and home frozen muscle.

The percent thaw drip from blast and home frozen roasts was calculated. To compare the effect of freezing rate on the weight of thaw drip released from blast and home frozen roasts, a paired Student's "t" test was computed (Steele and Torrie, 1960).

CHAPTER IV

RESULTS AND DISCUSSION

I. MYOGLOBIN

The myoglobin content of longissimus dorsi and psoas major muscles of fresh and frozen raw pork loin roasts is shown in Table I. The value of 0.47 mg per g of wet tissue for fresh raw pork longissimus dorsi was lower than the average value of 0.79 mg per g of wet tissue for light pork muscle reported by Ginger et al. (1954) and the average value of 0.86 mg per g of wet tissue for longissimus dorsi given by Janicki et al. (1967). Ginger et al. (1954) measured cyanmetmyoglobin; whereas, Janicki et al. (1967) measured carbonylmyoglobin. Both workers used unfrozen meat. Ginger et al. (1954) assumed a fifty percent partition between meat and extracts when one extraction was used. Janicki et al. (1967) used a double extraction; the present study assumed complete partition of the pigment with the use of a triple extraction. Unlike the present study, the earlier workers did not mention the use of a color classification system to select the meat investigated. The pork subjectively selected for the present study was lighter in color than average as shown by a lower than average color score of 2.5. Reporting the myoglobin values on a wet basis does not take into account the moisture and fat variations between animals.

The average value of 1.15 mg myoglobin per g wet tissue for fresh pork psoas major muscle was slightly lower than the average of 1.44 mg myoglobin per g of wet tissue for dark muscles reported by Ginger et al.

TABLE I
AVERAGE MYOGLOBIN CONTENT OF LONGISSIMUS DORSI AND PSOAS MAJOR
MUSCLES FROM RAW PORK LOIN ROASTS

Treatment	Myoglobin Content ^a			
	Fresh Basis (mg/g)		Fat-Free-Dry Basis (mg/g)	
	Longissimus Dorsi	Psoas Major	Longissimus Dorsi	Psoas Major
Fresh	0.47 ± 0.02	1.15 ± 0.07	2.08 ± 0.12	5.13 ± 0.32
Blast Frozen	0.64 ± 0.02	1.37 ± 0.13	2.58 ± 0.25	6.07 ± 0.59
Home Frozen	0.61 ± 0.06	1.33 ± 0.02	2.70 ± 0.29	5.82 ± 0.50

^a Mean and standard error of 15 or 16 values for fresh samples and 7 or 8 values for frozen samples.

(1954), and fell within the range of 1-3 mg myoglobin per g of wet tissue for pork muscles as given by Clydesdale and Francis (1971). The longissimus dorsi muscle was lower in myoglobin.

The averages of 0.64 and 0.61 mg myoglobin per g of wet tissue for frozen longissimus dorsi muscles, Table I, were greater than the average value of 0.43 mg myoglobin reported by Akin (1971) for frozen pork of a varying color range. The use of three extractions in the present study, in contrast to the two extractions of myoglobin by Akin (1971), could account for the higher average. The average values for frozen psoas major muscles, 1.37 and 1.33 mg myoglobin per g for wet tissue, fell within the range given by Clydesdale and Francis (1971).

The myoglobin contents for longissimus dorsi and psoas major muscles on a fat-free-dry basis are shown in Table I, also. The values of 2.58 and 2.70 mg myoglobin per g of fat-free-dry tissue for longissimus dorsi muscle, blast and home frozen, respectively, are slightly higher than the average value of 1.86 mg myoglobin per g fat-free-dry tissue of frozen longissimus dorsi reported by Akin (1971). The higher concentrations of 6.07 and 5.87 mg of myoglobin for psoas major muscles on a fat-free basis indicated that the myoglobin content was not a function of the fat content of the muscle. Refer to Tables XIII, XIV, XV, XVI (Appendix, pages 47, 48, 49, and 50) for the myoglobin content, percent fat-free-dry matter, percent fat and percent moisture, respectively, of each muscle analyzed. Marked variation in fat content between roasts from the same loin, as well as among loins, is evident in the data in Table XV (Appendix, page 49). The values in Table XV and Table XVI (Appendix, pages 49 and 50) are similar to

the average values for raw pork loin of medium fat class for fat, 11.4 percent, and moisture, 67.5 percent (Watt and Merrill, 1963).

The mean squares from the analysis of variance for myoglobin content of fresh and frozen longissimus dorsi and psoas major muscles are shown in Table II. As would be expected, the data indicated a significant ($P < 0.001$) difference between the two muscles. The average myoglobin content of the psoas major muscles was slightly more than twice as high as that for the longissimus dorsi, Table I (page 22). Ginger et al. (1954) observed a two-fold difference between the pigment content of light and dark pork muscles from the same cut.

A significant ($P < 0.01$) difference in myoglobin content was associated with freezing treatment. Results of a Duncan's New Multiple Range Test of these data appear in Table III. The test indicated that the average myoglobin content of frozen muscles was higher than that of the fresh muscles. No significant difference was found between blast frozen and home frozen muscles. A more efficient extraction of myoglobin from frozen tissue may account for the significant difference between fresh and frozen muscles. DeDuve (1948) noted that myoglobin was more easily extracted from frozen meat than from fresh. Thus, damage due to freezing may allow the myoglobin to be released more readily by the meat fibers. Since freezing decreases water-holding capacity of meat, frozen samples may release more moisture than the fresh samples during the extraction process.

A significant difference ($P < 0.001$) in myoglobin content was associated with carcass as indicated in Table II. The results of a Duncan's New Multiple Range Test of the data appear in Table IV. Even

TABLE II
MEAN SQUARE AND F VALUES FROM LEAST SQUARES ANALYSIS
OF VARIANCE FOR MYOGLOBIN CONTENT OF FRESH AND
FROZEN PORK ROASTS

Source	Degrees of Freedom	Mean Squares	F Values
Muscle	1	146.618	76.924***
Treatment	2	4.442	8.350**
Carcass	7	6.180	98.095***
Muscle x Treatment	2	0.320	5.079*
Muscle x Carcass ^a	7	1.906	
Treatment x Carcass ^b	14	0.532	
Muscle x Treatment x Carcass ^c	13	0.063	
Error ^d	15	0.063	

^aError term for Muscle.

^bError term for Treatment.

^cError term for Muscle x Treatment.

^dError term for Carcass.

*P < 0.05.

**P < 0.01.

***P < 0.001.

TABLE III

DUNCAN'S NEW MULTIPLE RANGE TEST FOR AVERAGE MYOGLOBIN
CONTENT OF ROASTS FRESH, BLAST FROZEN OR HOME FROZEN^a

Treatment	Fresh	Home Frozen	Blast Frozen
Treatment Means	3.65	4.17	4.33

^aMeans not underlined by the same line are significantly ($P < 0.05$) different. Values are means for both muscles expressed as mg Mb per g FFD tissue.

TABLE IV

DUNCAN'S NEW MULTIPLE RANGE TEST FOR AVERAGE
MYOGLOBIN CONTENT BY CARCASSES^a

Carcass Number	I	III	VI	VII	IV	II	VIII	V
Carcass Means	2.24	3.41	3.72	4.13	4.20	4.47	4.51	4.90

^aMeans not underlined by the same line are significantly ($P < 0.05$) different. Values are means of both muscles and three treatments expressed as mg Mb per g FFD tissue.

though carcasses were selected to fall within a narrow visual color range, there was considerable variation in myoglobin content.

The average weight of drip and total myoglobin content of thaw drip from frozen roasts is given in Table V. There was little drip from these roasts with either method of freezing, Table XVIII (Appendix, page 52). Total myoglobin released into the thaw drip was not significantly ($P > 0.05$) influenced by the rate of freezing. The effect of freezing rate may have been lessened by the rather large variation, as indicated by the standard error, in the weight of drip as well as in myoglobin content of the drip. The total myoglobin in the drip of each blast and home frozen roast appears in Table XVII (Appendix, page 51).

The percentage recovery of myoglobin in each frozen roast compared to the myoglobin in the paired fresh roast is shown in Table VI. In roasts from carcass I through V, about 100 percent recovery was obtained. In roasts VI through VIII, recovery exceeded 100 percent. Inspection of the data indicate that one of the lowest values for myoglobin was obtained with fresh roasts from carcass VI, Table XIII (Appendix, page 47); but relatively high values were obtained from the frozen muscles from this loin. It therefore appears that inefficient extraction of myoglobin from two of the fresh raw muscles was responsible for the high recovery. The values for myoglobin content of the drip in roasts VII and VIII are in line with the other roasts of the study. The roasts from carcass VI had less drip and therefore less myoglobin in the drip. The values in Table XIII (Appendix, page 47) would indicate that the high recoveries shown in Table VI were due to myoglobin in the muscles rather than the drip. Possibly the effect noted by DeDuve (1948) was active in the last three paired loins analyzed in this study.

TABLE V
MYOGLOBIN CONTENT OF THAW DRIP FROM FROZEN ROASTS

Treatment	Wt of Drip ^a (g)	Total Myoglobin Per Roast ^a (mg)
Blast Frozen	4.22 \pm 1.38	14.88 \pm 3.90 ^b
Home Frozen	6.61 \pm 1.47	22.46 \pm 4.69 ^b

^aMean and standard error of 7 or 8 samples.

^bNonsignificant difference between the means.

TABLE VI
RECOVERY OF MYOGLOBIN IN FROZEN ROASTS

Carcass Number	Blast Frozen			Home Frozen		
	Total Myoglobin		Recovery (%)	Total Myoglobin		Recovery (%)
	Fresh Roast (mg)	Frozen Roast + Drip (mg)		Fresh Roast (mg)	Frozen Roast + Drip (mg)	
I	2456.2	2127.5	86.6	2431.6	2421.8	99.5
II	4994.7	5184.4	103.7	3184.8	2998.6	94.1
III	--	--	--	4219.7	4284.7	101.5
IV	--	--	--	3006.6	3184.8	105.9
V	3417.2	3412.1	99.8	4088.9	4375.6	107.0
VI	2177.1	3900.0	179.1	2670.5	4692.2	175.7
VII	4542.2	5966.7	131.3	4247.1	6804.2	160.2
VIII	4156.1	5681.4	136.7	--	--	--
Mean \pm	3623.9	4378.7	122.9	3407.0	4108.8	120.6
Standard Error	465.6	608.2	13.7	290.1	548.6	12.4

II. COLOR REFLECTANCE

Mean CIE values for longissimus dorsi and psoas major muscles from raw pork loin roasts are shown in Table VII. The averages for dominant wavelength are within the orange to reddish orange regions of the chromaticity diagram. Akin (1971) analyzed frozen, thawed pork longissimus dorsi muscles with a Color Eye Colorimeter. In Akin's (1971) study, the average lightness index (L) for muscles in color class 2 was 5.990; for muscles in color class 3, 4.776. In the present study, the L value of 5.79 and 5.75 for blast and home frozen longissimus dorsi in color class 2.5 fell within Akin's reported range. As lightness index increases, the brightness of the sample color increases.

Mean squares from the analysis of variance for CIE values of longissimus dorsi and psoas major muscles from pork loin roasts appear in Table VIII. For dominant wavelength, a significant difference was associated with carcass. The results of the Duncan's New Multiple Range Test of these data appear in Table IX. The significant ($P < 0.05$) interaction between muscle and treatment indicated treatment did not affect all muscles in the same way, Table VIII.

For purity and lightness index, a highly significant ($P < 0.001$) difference was found between the two muscles but not among treatments or interactions. A significant ($P < 0.01$) difference among carcasses was found for both purity and lightness. The results of a Duncan's New Multiple Range Test of these data appear in Tables X and XI. The analysis indicates that the difference in color of longissimus dorsi and psoas major as seen by the human eye was influenced by purity and

TABLE VII
MEAN CIE VALUES OF LONGISSIMUS DORSI AND PSOAS MAJOR
MUSCLES FROM RAW PORK LOIN ROASTS

Muscle and Treatment	CIE Values ^a		
	Dominant Wavelength (nm)	Purity (%)	Lightness Index
Longissimus Dorsi			
Fresh	596 \pm 2	17 \pm 1	5.85 \pm 0.16
Blast Frozen	592 \pm 2	17 \pm 1	5.79 \pm 0.17
Home Frozen	603 \pm 5	16 \pm 1	5.75 \pm 0.16
Psoas Major			
Fresh	589 \pm 1	30 \pm 2	4.47 \pm 0.08
Blast Frozen	598 \pm 4	29 \pm 2	4.43 \pm 0.15
Home Frozen	599 \pm 4	29 \pm 2	4.35 \pm 0.17

^a Mean and standard error of 16 determinations for fresh and 8 for frozen.

TABLE VIII
MEAN SQUARES OF CIE VALUES FOR LONGISSIMUS DORSI
AND PSOAS MAJOR MUSCLES FROM RAW PORK LOIN
ROASTS FRESH AND FROZEN

Source	Degrees of Freedom	Mean Squares ^a		
		D W	P	L
Muscle	1	77.006	2131.600***	27.176***
Treatment	2	318.797	8.461	0.063
Carcass	7	121.785*	81.129**	0.889**
Muscle x Treatment	2	272.547*	4.148	0.004
Muscle x Carcass ^b	7	46.249	28.857	0.201
Treatment x Carcass ^c	14	147.404	15.818	0.114
Muscle x Treatment x Carcass ^d	14	35.404	15.791	0.069
Error ^e	16	40.344	18.875	0.085

^aD W is dominant wavelength; P is purity; L is lightness index.

^bError term for Muscle.

^cError term for Treatment.

^dError term for Muscle x Treatment.

^eError term for Carcass.

*P < 0.05.

**P < 0.01.

***P < 0.001.

TABLE IX
DUNCAN'S NEW MULTIPLE RANGE TEST FOR AVERAGE DOMINANT
WAVELENGTH BY CARCASSES^a

Carcass Number	V	VIII	II	I	III	IV	VII	VI
Carcass Means	592	592	593	595	596	596	598	600

^aMeans not underlined by the same line are significantly ($P < 0.05$) different.

TABLE X
DUNCAN'S NEW MULTIPLE RANGE TEST FOR AVERAGE
COLOR PURITY BY CARCASSES^a

Carcass Number	I	VIII	VI	III	VII	II	V	IV
Carcass Means	18	20	21	22	24	25	26	27

^aMeans not underlined by the same line are significantly ($P < 0.05$) different.

TABLE XI
DUNCAN'S NEW MULTIPLE RANGE TEST FOR AVERAGE LIGHTNESS
INDEX BY CARCASSES^a

Carcass Number	V	III	II	VII	IV	VI	VIII	I
Carcass Means	4.98	5.01	5.03	5.12	5.16	5.20	5.24	5.81

^aMeans not underlined by the same line are significantly ($P < 0.05$) different.

lightness index but not by dominant wavelength. Freezing and thawing did not significantly affect any of the three CIE values tested.

Lentz (1971) reported similar results from a study of blast frozen (-20°F) beef muscles stored (0° to -40°F) in the dark and measured with Munsell color chips. Frozen, thawed samples did not differ in hue from the fresh samples. Chroma decreased for all samples during storage and value remained constant. Significance of differences was not reported by Lentz (1971).

III. INTERRELATIONS OF MYOGLOBIN CONTENT WITH CIE VALUES

Correlation coefficients of myoglobin content and CIE values for longissimus dorsi and psoas major muscles from pork loin roasts fresh and frozen are shown in Table XII. As compared with fresh muscles, the degrees of freedom for frozen muscles were limited by a fewer number of samples. A significant negative correlation ($r = -0.584$) was found between dominant wavelength and myoglobin content of fresh psoas major muscle. As myoglobin content increased, dominant wavelength decreased. In the study, this was the only muscle in which there was a significant relationship between myoglobin and dominant wavelength. The lack of correlation between dominant wavelength and myoglobin in other cases may be partially explained by the significant interaction between muscle and treatment as shown in Table VIII (page 32).

Correlations between purity and pigment content were significant or approaching significance. This indicates that as myoglobin content increased, purity increased.

Negative significant correlations or correlations approaching

TABLE XII
CORRELATIONS OF MYOGLOBIN CONTENT WITH COLOR REFLECTANCE VALUES
OF MUSCLES FROM PORK LOIN ROASTS

Treatment and Muscle	Degrees of Freedom	Comparisons ^a	"r"
Fresh, Longissimus Dorsi	13	Mb vs. D W	-.209
	13	Mb vs. P	+.420
	13	Mb vs. L	-.571*
Fresh, Psoas Major	14	Mb vs. D W	-.584 [†]
	14	Mb vs. P	+.574
	14	Mb vs. L	-.540*
Blast Frozen, Longissimus Dorsi	6	Mb vs. D W	+.100 [†]
	6	Mb vs. P	+.807
	6	Mb vs. L	-.561
Blast Frozen, Psoas Major	6	Mb vs. D W	+.096
	6	Mb vs. P	+.498
	6	Mb vs. L	-.562
Home Frozen, Longissimus Dorsi	6	Mb vs. D W	+.563
	6	Mb vs. P	+.344
	6	Mb vs. L	-.537
Home Frozen, Psoas Major	5	Mb vs. D W	+.312
	5	Mb vs. P	+.569
	5	Mb vs. L	-.573

^aMb is myoglobin; D W is dominant wavelength; P is purity; L is lightness index.

* $P < 0.05$.

[†] $P < 0.025$.

significance were consistently shown between lightness index and myoglobin content. As would be expected, color lightness increased with decreased pigment content. The nonsignificant correlations of myoglobin content and CIE values may indicate that the color of meat as seen by the human eye is influenced by other parameters as well as by myoglobin content.

IV. FREEZING AND FREEZING RATE EFFECT ON THAW DRIP

The amount and percentage of drip from blast and home frozen pork loin roasts are shown in Table XVIII (Appendix, page 52). The weight of drip from some of the roasts was so small that quantitative measurement was very difficult. A trend was not detected between weight of thaw drip and weight of roast, fat-free-dry matter of tissue, percent fat or percent moisture, Tables XVIII, XIV, XV, XVI (Appendix, pages 52, 48, 49, and 50). The weight of drip released during thawing varied with the individual roasts as well as with the rate of freezing. However, a significant ($P < 0.05$) difference was found between the amount of thaw drip lost from blast and home frozen roasts, the slowest rate of freezing resulting in more drip. The drip from the home frozen roasts averaged 50 percent more than the drip from the blast frozen roasts. Ramsbottom and Koonz (1941) found steaks frozen at 10°F had 52 percent greater loss in drip than steaks which were frozen at -30°F .

V. RECOMMENDATIONS

A pure myoglobin standard to test the reliability of the chemical method used is a need for further research. In the present study,

attempts were made to do a standard recovery using horse heart muscle myoglobin but there was too much insoluble material to obtain an accurate weight of standard. Additional work with freezing effects on myoglobin content and color reflectance of pork would be informative. Valuable information for industry could be gathered by investigating the relationship between consumer acceptance, myoglobin content, and color reflectance of frozen meat.

CHAPTER V

SUMMARY

I. SCOPE OF STUDY

The purpose of this study was to investigate the effect of freezing rate on myoglobin content of thawed pork loin roasts and of the thaw drip. The relationship of myoglobin content to color reflectance was also studied. Roasts from eight pairs of pork loins had a color score of 2.5 on a 5 point scale. Of the 32 roasts, one of each pair was analyzed fresh and the other analyzed after freezing and thawing. Freezing was done at one of two rates: in an air blast freezer at -27° to -29°C and in a home freezer at -18°C . Roasts were stored for 28 days before thawing.

Myoglobin was measured chemically on longissimus dorsi and psoas major muscles from each of 32 roasts, and on the thaw drip from the frozen roasts. Color reflectance of both muscles was measured spectrophotometrically utilizing the Color Eye Colorimeter.

II. PRINCIPAL FINDINGS

The myoglobin content of longissimus dorsi and psoas major muscles was significantly ($P < 0.001$) different. The myoglobin content of the psoas major was approximately twice that of the longissimus dorsi muscle. Significantly ($P < 0.01$) less myoglobin was found in fresh than frozen, thawed roasts; but no difference was found between roasts frozen at the two rates. A more efficient myoglobin extraction of frozen

muscles than fresh muscles was proposed. The myoglobin content among carcasses was significantly ($P < 0.001$) different. No differences were detected in total myoglobin released into the thaw drip of blast and home frozen roasts. Recovery of myoglobin in frozen roasts averaged 120 percent.

Dominant wavelength for longissimus dorsi and psoas major muscles differed significantly ($P < 0.05$) among carcasses. Significant differences between muscles and among carcasses were shown with both lightness index and purity.

With fresh pork muscles, purity was positively correlated with myoglobin content; lightness index was negatively correlated. Significant correlations between CIE values and myoglobin content of frozen muscles were not found.

Significantly ($P < 0.05$) more drip was released from home than blast frozen roasts.

LIST OF REFERENCES

LIST OF REFERENCES

- Akin, W. A. 1971. Sensory and objective evaluation of the color of pork longissimus dorsi muscle heated to 155° and 170°F. M.S. thesis, The University of Tennessee, Knoxville, Tn.
- Anonymous. 1969. Meat freezing. In "Homemakers Opinions About Selected Meats, A Nationwide Survey" Marketing Research Report 854, USDA, Washington, D. C.
- Anonymous. 1972. Frozen foods will grow 53.1% to a total value of \$12.3 billion. Quick Frozen Foods 14:57.
- Aref, M. M. 1965. The present status of liquid nitrogen freezing of meat. Can. Inst. Food Technol. J. 1:11.
- Beecher, G. R., Cassens, R. G., Hoekstra, W. G., and Briskey, E. J. 1965. Red and white fiber content and associated post-mortem properties of seven porcine muscles. J. Food Sci. 30:969.
- Bodwell, C. E. and McClain, P. E. 1971. Chemistry of animal tissue proteins. In "The Science of Meat and Meat Products", ed. Price, J. F. and Schweigert, B. S. pp. 371-373, 2nd Ed. W. H. Freeman and Co., San Francisco.
- Borgstrom, G. 1968. "Principles of Food Science". Vol. 1, pp. 210-211 and 220. Macmillan Co., New York.
- Bratzler, L. J. 1968. The preparation for freezing and freezing of meats. In "The Freezing Preservation of Foods", ed. Tressler, D. K., Van Arsdell, W. B., Copley, M. J. Vol. 3, p. 202. The AVI Publishing Co., Inc., Westport, Conn.
- Briskey, E. J. and Kauffman, R. G. 1971. Quality characteristics of muscle as a food. In "The Science of Meat and Meat Products", ed. Price, J. F. and Schweigert, B. S. pp. 371-373, 2nd Ed. W. H. Freeman and Co., San Francisco.
- Cassens, R. G. 1971. Microscopic structure of animal tissues. In "The Science of Meat and Meat Products", ed. Price, J. F. and Schweigert, B. S. pp. 63-67, 2nd Ed. W. H. Freeman and Co., San Francisco.
- Clydesdale, F. M. and Francis, F. J. 1971. The chemistry of meat color. Food Product Development 5:81.
- Crandall, M. W. and Drabkin, D. L. 1946. Cytochrome c in regenerating rat liver and its relation to other pigments. J. Biol. Chem. 166:653.

- DeDuve, C. 1948. A spectrophotometric method for the simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. *Acta Chem. Scand.* 2:264.
- Doty, D. M. 1960. Physical methods. In "The Science of Meat and Meat Products", American Meat Institute Foundation. pp. 232-233. W. H. Freeman and Co., San Francisco.
- Drabkin, D. L. and Austin, J. H. 1935. Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *J. Biol. Chem.* 112:51.
- Drabkin, D. L. 1950. The distribution of the chromoproteins, hemoglobin, myoglobin, and cytochrome c in the tissues of different species, and relationship of the total content of each to body mass. *J. Biol. Chem.* 182:317.
- Ewing, G. W. 1960. "Instrumental Methods of Chemical Analysis". p. 26, 2nd Ed. McGraw-Hill Book Co., New York.
- Fleming, H. P., Blumer, T. N. and Craig, H. B. 1960. Quantitative estimations of myoglobin and hemoglobin in beef muscle extracts. *J. Animal Sci.* 19:1164.
- Ginger, I. D., Wilson, G. D. and Schweigert, B. S. 1954. Biochemistry of myoglobin. Quantitative determination in beef and pork muscle. *J. Agr. Food Chem.* 2:1037.
- Harvey, W. R. 1960. Least squares analysis of data with unequal subclass numbers. Agriculture Research Service 20-8, USDA, Beltsville, Maryland.
- Hiner, R. L. and Hankins, O. G. 1947. Temperatures of freezing affects tenderness of beef. *Food Industries* 19:1078.
- Iowa State University. 1969. Standards for pork color, firmness, and marbling. Publ. Pm-452. Cooperative Extension Service. Ames, Iowa.
- Janicki, M. A., Kortz, J. and Rozyozka, J. 1967. Relationship of color with certain chemical and physical properties of porcine muscle. *J. Food Sci.* 32:375.
- Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* 12:307.
- Kendrew, J. C. 1963. Myoglobin and the structure of proteins. *Science* 139:3561.
- Koonz, C. H. and Ramsbottom, J. H. 1939. A method for studying the histological structure of frozen products. I. Poultry. *Food Res.* 4:117.

- LaClerq, E. L. 1970. Mean separation by the functional analysis of variance and multiple comparisons. Agricultural Research Service 20-3, USDA, Beltsville, Maryland.
- Lawrie, R. A. 1950. Some observations of factors affecting myoglobin concentration in muscle. J. Ag. Sci. 40:356.
- Ledward, D. A. 1970. Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. J. Food Sci. 35:33.
- Lentz, C. P. 1971. Effect of light and temperature on color and flavor of prepackaged frozen beef. J. Inst. Can. Technol. Aliment. 4:166.
- Mackinney, G. and Little, A. C. 1962. "Color of Foods". pp. 265-266 and 278-279. The AVI Publishing Co., Westport, Conn.
- Moody, W. G. and Cassens, R. G. 1968. Histochemical differentiation of red and white muscle fibers. J. Animal Sci. 27:961.
- Morgan, V. E. 1936. Studies on myoglobin I. The solubility of myoglobin in concentrated ammonium sulfate solutions. J. Biol. Chem. 112:557.
- Paul, P. C. 1972. Meat. In "Food Theory and Application", ed. Paul, P. C. and Palmer, H. H. p. 335. John Wiley and Sons, Inc., New York.
- Poel, W. E. 1949. Effect of anoxia on myoglobin concentration in striated muscle. Amer. J. Physiol. 156:44.
- Rickansrud, D. A. and Henrickson, R. L. 1967. Total pigments and myoglobin concentration in four bovine muscles. J. Food Sci. 32:52.
- Ramsbottom, J. M. and Koonz, C. H. 1941. Freezer storage temperature as related to drip and to color in frozen defrosted beef. Food Res. 6:571.
- Shenk, J. H., Hall, J. L. and King, H. H. 1934. Spectrophotometric characteristics of hemoglobin. I. Beef blood and muscle hemoglobin. J. Biol. Chem. 105:741.
- Solberg, M. 1970. The chemistry of color stability in meat--a review. Can. Inst. Food Technol. J. 3:55.
- Steele, R. G. D. and Torrie, J. H. 1960. "Principles and Procedures of Statistics". McGraw-Hill Book Co., Inc., New York.
- Theorell, A. H. T. 1932. Kristallsieren und reinerung des myoglobins sowie vorlaufige mitteilung uber sein molekulargewicht. Biochem. Z. 252:1.

- Tuma, H. J. 1971. Processing technology for freezing retail meat cuts. In "Proceedings of the Meat Industry Research Committee". p. 62. American Meat Institute Foundation, Chicago.
- van der Oord, A. H. A. and Wesdorp, J. J. 1971. Analysis of pigments in intact beef samples. J. Food Technol. 6:1.
- Watt, B. K. and Merrill, A. L. 1963. Composition of food. Agricultural Handbook 8, p. 48. Consumer and Food Economics Research Division, ARS, USDA, Washington, D. C.

APPENDIX

APPENDIX

TABLE XIII

MYOGLOBIN IN LONGISSIMUS DORSI AND PSOAS MAJOR MUSCLES OF
RAW PORK LOIN ROASTS FRESH AND BLAST OR HOME FROZEN

Carcass Number	Myoglobin Content (mg/g fat-free-dry tissue)							
	Fresh		Blast Frozen		Fresh		Home Frozen	
	Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major
I	1.30	3.14	0.98	3.09	1.24	3.47	1.23	3.48
II	2.60	5.99	2.69	6.55	2.42	6.48	2.72	6.33
III	--	4.23	2.17	4.59	2.02	4.23	1.99	4.63
IV	1.88	5.41	2.97	6.18	2.62	5.49	2.90	6.14
V	2.42	7.40	2.69	7.49	2.48	6.59	2.62	7.54
VI	1.60	4.16	3.08	6.30	1.72	3.76	2.92	6.19
VII	2.39	4.67	3.18	5.79	2.26	4.55	3.81	6.41
VIII	2.12	6.38	2.88	8.57	2.10	6.07	3.47	--

TABLE XIV

PERCENT FAT-FREE-DRY MATTER FOR LONGISSIMUS DORSI AND PSOAS MAJOR
MUSCLES OF RAW PORK LOIN ROASTS FRESH AND
BLAST OR HOME FROZEN

Carcass Number	Fresh		Percent Fat-Free-Dry Matter				Home Frozen	
	Long. Dorsi	Psoas Major	Blast Frozen		Fresh		Long. Dorsi	Psoas Major
			Long. Dorsi	Psoas Major	Long Dorsi	Psoas Major		
I	23.2	24.8	24.2	24.0	24.3	23.2	24.8	23.5
II	22.2	23.6	23.5	23.5	19.3	21.7	21.3	23.5
III	23.3	23.8	24.7	23.5	22.9	23.0	23.8	23.2
IV	23.9	23.1	24.0	23.8	23.0	23.0	24.0	24.7
V	23.8	21.0	22.6	22.6	21.8	22.3	22.7	23.2
VI	23.2	21.1	20.4	20.4	22.8	22.1	22.2	20.4
VII	23.2	21.5	22.2	21.8	21.4	21.8	20.0	21.9
VIII	24.3	22.5	22.0	22.2	21.3	21.8	23.2	22.7
Mean \pm	23.4	22.7	23.0	22.7	22.1	22.4	22.8	22.9
Standard Error	0.2	0.5	0.5	0.4	0.5	0.2	0.6	0.4

TABLE XV

FAT CONTENT OF LONGISSIMUS DORSI AND PSOAS MAJOR MUSCLES
OF RAW PORK LOIN ROASTS FRESH AND BLAST OR HOME FROZEN

Carcass Number	Percent Fat							
	Fresh		Blast Frozen		Fresh		Home Frozen	
	Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major
I	4.2	3.4	4.5	4.3	6.8	3.7	7.2	5.2
II	10.7	4.5	11.8	4.6	18.1	6.9	17.1	4.9
III	3.6	1.3	6.7	1.1	3.0	1.2	6.7	1.5
IV	3.4	2.5	3.4	2.2	4.6	2.1	4.6	2.3
V	9.9	5.8	12.0	5.0	10.4	3.9	8.0	4.1
VI	9.3	3.5	11.8	5.1	4.8	3.9	4.1	5.1
VII	9.5	2.6	7.7	2.7	8.7	2.2	14.7	3.3
VIII	6.0	3.2	11.9	3.6	13.6	3.6	9.0	3.3
Mean \pm	7.1	3.4	8.7	3.6	8.8	3.4	8.9	3.7
Standard Error	1.1	0.5	1.3	0.5	1.8	0.6	1.6	0.5

TABLE XVI

MOISTURE CONTENT OF LONGISSIMUS DORSI AND PSOAS MAJOR MUSCLES
OF RAW PORK LOIN ROASTS FRESH AND BLAST OR HOME FROZEN

Carcass Number	Percent Moisture							
	Fresh		Blast	Frozen	Fresh		Home Frozen	
	Long. Dorsi	Psoas Major			Long. Dorsi	Psoas Major	Long. Dorsi	Psoas Major
I	72.5	71.8	71.3	71.7	69.2	73.4	68.0	71.3
II	67.1	71.9	64.7	71.9	62.6	71.3	61.6	71.6
III	73.8	74.9	68.6	75.4	73.5	75.8	69.4	75.3
IV	72.7	74.4	72.5	74.0	72.4	74.9	71.3	72.9
V	68.3	73.2	65.3	72.5	65.7	73.8	69.3	72.7
VI	67.4	75.4	67.8	74.5	72.2	74.0	72.0	74.4
VII	69.1	75.6	70.1	75.5	68.0	76.3	65.3	74.7
VIII	69.6	74.0	66.1	74.2	65.1	74.6	67.8	73.9
Mean \pm	70.1	73.9	68.3	73.7	68.6	74.3	68.1	73.4
Standard Error	0.9	0.5	1.0	0.5	1.4	0.5	1.2	0.5

TABLE XVII
TOTAL MYOGLOBIN IN THE DRIP OF BLAST
AND HOME FROZEN ROASTS

Carcass Number	Total Myoglobin Content (mg)	
	Blast Frozen	Home Frozen
I	26.32	26.49
II	16.84	14.76
III	5.88	13.38
IV	--	31.87
V	21.55	24.11
VI	0.03	0.09
VII	25.54	44.34
VIII	7.65	24.66
Mean \pm	14.88	22.46
Standard Error	3.90	4.69

TABLE XVIII
AMOUNT AND PERCENTAGE OF DRIP FROM BLAST AND HOME FROZEN PORK LOIN ROASTS

Carcass Number	Blast Frozen				Home Frozen			
	Wt of Roast (g)	Time to Reach -4°C (min)	Wt of Drip (g)	Percent Drip	Wt of Roast (g)	Time to Reach -4°C (min)	Wt of Drip (g)	Percent Drip
I	669.6	176	10.98	1.64	906.3	196	10.33	1.14
II	650.0	140	2.30	0.35	641.3	624	1.78	0.28
III	657.0	60	0.75	0.11	619.1	576	4.18	0.68
IV	569.0	124	3.74	0.66	673.2	400	9.99	1.48
V	587.1	112	5.75	0.98	540.5	536	10.88	2.01
VI	836.3	184	0.22	0.03	697.2	632	0.46	0.07
VII	713.4	168	8.55	1.20	905.3	556	9.65	1.07
VIII	901.2	160	1.50	0.17	716.8	584	5.62	0.78
Mean \pm	698.0	140	4.22*	0.64	712.1	513	6.61*	0.94
Standard Error	41.0	14	1.38	0.21	46.0	52	1.47	0.22

*P < 0.05.

CALCULATIONS FOR CIE VALUES

$$X_{cie} = X_{ce}(0.6633) + X'_{ce}(0.1711)$$

$$Y_{cie} = Y_{ce}(0.8560)$$

$$Z_{cie} = Z_{ce}(1.0109)$$

$$\text{Coordinate } x = \frac{X_{cie}}{X_{cie} + Y_{cie} + Z_{cie}}$$

$$\text{Coordinate } y = \frac{Y_{cie}}{X_{cie} + Y_{cie} + Z_{cie}}$$

$$\text{Lightness Index} = \sqrt{Y_{ce}}$$

The dominant wavelength is the wavelength which passes through both the intersect of x and y and the Illuminant C.

Purity is determined on the chromaticity diagram by measuring the relative distances of the intersect of x and y and the corresponding spectrum point from the Illuminant C. Purity is expressed in percentage from 1 to 100 percent.

cie = CIE notation

ce = reading from Color Eye Colorimeter

VITA

Jane Shellabarger Nocito was born on May 10, 1949, in Dayton, Ohio. She attended elementary and high school in Trotwood, Ohio, where she graduated from the Madison Township School System in 1967. In the fall, she entered Ohio University and received a Bachelor of Science in Home Economics in June of 1971.

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