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Myoglobin Concentration of Turkey Muscle as Affected by Heat

Janice Gail Sandefer
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Janice Gail Sandefer entitled "Myoglobin Concentration of Turkey Muscle as Affected by Heat." 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.

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I am submitting herewith a thesis written by Janice Gail Sandefer entitled "Myoglobin Concentration of Turkey Muscle as Affected by Heat." 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.

Major Professor

We have read this thesis
and recommend its acceptance:

Jane R. Savage

Bernadine Meyer

Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

MYOGLOBIN CONCENTRATION OF TURKEY MUSCLE AS AFFECTED BY HEAT

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Janice Gail Sandefer

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ABSTRACT

The effect of heat on myoglobin concentration of pectoralis major and thigh muscles of nine turkey toms was studied. Heat treatments included end point temperatures of 35°, 50°, and 65°C.

Myoglobin concentration of the pectoralis major as expressed on a wet and a nonfat dry basis did not change with increasing temperature. However, myoglobin concentration in the thigh on both a wet and a nonfat dry weight basis decreased as temperature increased ($P < 0.005$). The concentration of the pigment did not decrease until the samples were cooked to 50°C, and some myoglobin was still present at 65°C. Myoglobin concentration of the thigh was higher than that of the pectoralis major on both a wet and a nonfat dry basis ($P < 0.001$).

Moisture decreased in both the pectoralis major and thigh as temperature increased ($P < 0.005$) and tended to be higher in the thigh than in the pectoralis major for the raw and 35°C samples. Fat also decreased in the pectoralis major as temperature increased ($P < 0.05$) but, although higher in the thigh than in the pectoralis major, was unrelated to temperature.

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CHAPTER I

INTRODUCTION

Myoglobin concentration of muscle tissue has been determined for a variety of animals, some of which are used for food and others that are not. Some factors studied that affect myoglobin concentration have included age, activity, species, strain, and sex. Since myoglobin is mainly protein in structure, it will be affected by heat. Little attention has been given to the effect of heat treatment on myoglobin concentration, and this is the major interest in this investigation.

Myoglobin is a respiratory pigment primarily responsible for the color of meat. It is a porphyrin-protein complex composed of the heme portion, or an iron atom bound in the porphyrin ring, to which a globin portion is complexed.

Although myoglobin is the predominant pigment in meat, much more work has been reported on hemoglobin, possibly because myoglobin was more difficult to obtain in a sufficiently purified form than was hemoglobin. Reliable information concerning the properties of myoglobin was not available until its crystallization by Theorell in 1932 (Watts, 1954). Morgan (1936) in working with horse heart observed that the pigment was extremely soluble in phosphate buffers at pH 6.6 even at phosphate concentrations as high as 3 M. Since hemoglobin was not soluble under these conditions, this observation made possible the

introduction of a method for an almost quantitative separation of myoglobin and hemoglobin.

Myoglobin as a porphyrin-protein complex can undergo denaturation. Heat is one of the most common methods of denaturing protein in meat. Little work has been done on denaturation of the pigment especially as occurring in poultry. Because published reports of studies on thermal denaturation of myoglobin are limited, more research is needed to investigate the effect of temperature on the pigment. The purpose of this research was to study the effect of selected heat treatments on the myoglobin concentration of the pectoralis major muscle and of the thigh muscles of turkey.

CHAPTER II

REVIEW OF LITERATURE

I. MEAT COMPONENTS

Meat is composed of muscle fibers, connective tissue, fat, and bone. The essential unit of the muscle tissue is the fiber consisting of the myofibrils, between which is the sarcoplasm and a fine network of tubules, the sarcoplasmic reticulum. The fiber is bounded by a thin membrane, the sarcolemma. Chemically, meat is composed of 75.5% water, 18% protein, 3.5% soluble non-protein substances, and 3.0% fat (Lawrie, 1966).

The most abundant muscle protein is actomyosin, a complex of actin and myosin, which is responsible for the contractile properties of muscle. Edible muscle tissue also contains small amounts of (1) connective tissue proteins, collagen, elastin, and reticulin; (2) respiratory pigments, the most abundant of which is myoglobin; (3) nucleoproteins, the major constituent of genetic material in the cells; (4) enzymes, which function as biological catalysts in almost every chemical reaction within the cell; and (5) other proteins with miscellaneous functions (American Meat Institute Foundation, 1960).

II. MEAT PIGMENTATION

Compounds contributing to color in muscle tissue include myoglobin, hemoglobin, cytochromes, vitamin B_{12}, and the flavins. The heme

pigments, myoglobin, hemoglobin, and cytochromes, contain iron in a porphyrin-protein complex structure. Vitamin B_{12}, a much more complex structure but less important to pigmentation, contains the same porphyrin ring as the heme compounds, but has a cobalt atom instead of iron. The flavins, also of lesser importance to color of muscle tissue, are yellow coenzymes involved with cytochromes in electron transport in the cell.

Myoglobin is the pigment primarily responsible for the meat color. In the live animal, myoglobin accounts for about 10% of the iron. During the slaughter the bleeding process removes most of the iron in the form of hemoglobin. In well-bled skeletal muscle of beef as much as 95% or more of the iron present is in the form of myoglobin (American Meat Institute Foundation, 1960).

Myoglobin Structure and Function

Myoglobin is a porphyrin-protein complex composed of the heme portion, or an iron atom bound in a porphyrin ring, to which a globin portion is complexed. The porphyrin is made up of four pyrrole units, a heterocyclic compound linked by methene bridges. The three different kinds of side chains attached to the porphyrin portion of the molecule are methyl, vinyl, and propyl (American Meat Institute Foundation, 1960). The globin portion is a single polypeptide chain of 150 amino acid residues (Kendrew, 1963).

The muscle pigment is similar in function to the blood pigment hemoglobin in that they both complex with oxygen, although they differ in their roles. Hemoglobin serves as an oxygen carrier in the blood

stream, whereas myoglobin is basically a storage mechanism for oxygen in the cells.

Myoglobin's storage role is reflected in the quantities of the pigment found in various tissues. The quantity present is generally a function of the muscular activity of the tissue, the blood supply, the oxygen availability, and the age of the animal. For example (American Meat Institute Foundation, 1960), although myoglobin exists in small amounts in heart muscle, it is present in this organ in larger quantities than in any other tissue. Bird wing muscles with a highly efficient blood vessel system for supplying the increased demand of oxygen contain less myoglobin than other skeletal muscles in birds. Whales have the highest myoglobin content of all mammals which accounts for their ability to remain submerged for periods as long as an hour without breathing. The relationship between age of the animal and the myoglobin concentration is directly proportional. As the animal becomes older, the myoglobin content increases.

Myoglobin Forms and Characteristic Colors

In accordance with its ability to combine with oxygen, myoglobin has characteristic colors. Myoglobin, with the iron in the reduced state, has a purplish red color. Oxygenation of the pigment produces a bright red colored compound, oxymyoglobin, with the iron still in the reduced state. Metmyoglobin, with the iron in the oxidized state, is formed by oxidation of either myoglobin or oxymyoglobin and is brown in color. In meat there is a constant conversion of myoglobin and

oxymyoglobin to metmyoglobin. There is also a continuous supply of reducing coenzymes from the enzymatic oxidation of substrates such as glucose, which are capable of reducing metmyoglobin back to oxymyoglobin and myoglobin. Thus, on the surface of fresh meat as long as the supply of oxygen and reducing substances is plentiful, oxymyoglobin is evidenced by a bright red color. In the internal portion of the meat, the iron is in the reduced state, and myoglobin has a typical reddish purple color (American Meat Institute Foundation, 1960).

Myoglobin can react with substances other than oxygen and has other characteristic colors. Combination of myoglobin with nitric oxide produces nitric oxide myoglobin, in which the iron is in the reduced state, and has a characteristic red color. A combination of metmyoglobin with excess nitrite produces a red pigment, metmyoglobin nitrite, with the iron in the oxidized state. A green color is characterized by sulfmyoglobin and choleglobin. Sulfmyoglobin results from the effect of hydrogen sulfide and oxygen on myoglobin, and choleglobin is the resulting effect of hydrogen peroxide on myoglobin or oxymyoglobin (Lawrie, 1966).

Myoglobin Concentration

Factors affecting myoglobin concentration. Some factors affecting myoglobin concentration include age, activity, species, and sex. The effect of age and activity on myoglobin concentration in horse and pork muscles was investigated by Lawrie (1950). Horses were studied at three activity levels, and those with the highest degree of

activity had the highest concentration of myoglobin. Myoglobin concentration also increased as age increased ($P < 0.001$). The rate of increase was rapid from birth until after two years in the horse and up to one year in the pig. After these periods of time, the myoglobin remained constant except in the horse psoas major and the diaphragm which had a slow but significant rise throughout life. The effect of age, sex, and strain on myoglobin concentration also was indicated in a study of light and dark meat of turkey (Froning et al., 1968). Myoglobin increased with an increase in age ($P < 0.05$), with exception of light meat from hens. Toms had a higher myoglobin concentration ($P < 0.05$) than the hens, and strain also affected myoglobin with two of the five strains studied having a concentration lower than the other three.

A difference in myoglobin concentration related to species was found by Ginger et al. (1954) in quantitative determinations of myoglobin in beef and pork muscle. The ratio of the concentration in beef (3.7 mg./g. of tissue) to that of pork averaged 4.7 to 1 for light colored pork muscle (0.79 mg./g. of tissue) and 2.6 to 1 for dark-colored pork (1.44 mg./g. of tissue). The overall average myoglobin concentration of 2.43 mg./g. of wet tissue for beef muscle reported by Fleming et al. (1960) was somewhat lower than the 3.7 mg. reported by Ginger et al. (1954). In four beef muscles studied by Rickansrud et al. (1967), myoglobin concentration was highest in the biceps femoris followed by the longissimus dorsi and psoas major, and lowest in the semitendinosis.

Methodology for myoglobin determination. Although myoglobin is the predominant pigment in meat, much more work has been reported on hemoglobin, possibly because myoglobin was more difficult to obtain in a sufficiently purified form than hemoglobin. Reliable information concerning the properties of myoglobin was not available until its crystallization by Theorell in 1932 (Watts, 1954). According to Morgan (1936) Theorell's work with horse heart myoglobin proved that there was a protein resembling, but nevertheless distinct from, the blood pigment hemoglobin, a fact which prior to that time was in doubt. Morgan also reported that Theorell's procedure consisted essentially of extraction of the finely ground meat tissue with water, followed by precipitation with lead acetate. The filtrate then was repeatedly dialyzed against solutions of saturated ammonium sulfate until characteristic fan-shaped clusters of needle crystals appeared. In his work with solubility of horse heart myoglobin in concentrated ammonium sulfate solutions, Morgan (1936) observed that myoglobin appeared to be extremely soluble in phosphate buffers at pH 6.6, even at phosphate concentrations as high as 3 M. This observation resulted in a relatively simple method for almost quantitative separation of hemoglobin and myoglobin since hemoglobin was not soluble under these conditions.

Spectrophotometric studies on hemoglobin from washed blood cells were reported by Drabkin et al. (1935). Total pigment was determined by measuring the absorbance of cyanmethemoglobin in solution to which potassium ferricyanide had been added to provide a final ferricyanide

concentration of 0.6 mM./l., and sodium cyanide had been added to a final cyanide concentration of 0.8 mM./l. In the formula for conversion of optical density readings at 540 mμ to mM./l., an extinction coefficient of 11.5×10^3 for cyanmethemoglobin was used. Crandall et al. (1946) used Morgan's modification of Theorell's procedure in determining myoglobin content of rat muscle tissue. An extinction coefficient of 11.3×10^3 at a wavelength of 540 mμ was used. Ginger et al. (1954) in chemical studies with purified metmyoglobin from beef and pork muscle used a comparable method and used the molar extinction coefficient of 11.5×10^3 as that indicated by Drabkin et al. (1935) for cyanmethemoglobin. In a much later study of quantitative estimation of myoglobin in beef muscle, Fleming et al. (1960) used a molar extinction coefficient of 11.3×10^3 for cyanmetmyoglobin at a wavelength of 540 mμ. All these studies used the method for quantitative determination that began with Theorell, indicating that the method was applicable to a variety of species of animals.

III. PROTEIN DENATURATION

Tanford (1968) defined protein denaturation as a major change from the original native structure without alteration of the amino acid sequence, that is it may involve any modification of secondary, tertiary, or quaternary structure (Joly, 1965). The secondary structure involves the spiral peptide helixes stabilized by hydrogen bonding between carbonyl and imido groups; the tertiary, extensive coiling or

folding of the spiral chains into globular forms; and the quaternary, the polymerized units (Conn et al., 1966). Therefore, denaturation involves an unfolding or uncoiling of the straight chain portion of the molecule. Denaturation can be achieved by a variety of means, including heat, enzymatic activity, and pH. Slight changes in pH, either increasing or decreasing, may lead to unfolding of the protein molecule.

Heat is generally the cause of denaturation of protein in muscle tissue systems. The rate of most chemical reactions may be increased by a factor of two or three for each 10°C rise in temperature, whereas the rate of denaturation may increase by a factor of 600 for each 10°C rise in temperature (Fox et al., 1957). The effect of heat on solubility of globular and structural proteins of beef muscle was studied by Hamm et al. (1960). A marked decrease of the solubility of structural proteins was noted when meat was heated from 20° to 40°C, and an even greater decrease in solubility was observed between 40° and 60°C. Beyond 60°C the structural proteins were almost insoluble. Hamm (1966) postulated that between 35° and 50°C the actomyosin molecules were unfolded. This was accompanied by aggregation of the unfolded molecules and formation of new, relatively unstable cross linkages. The unfolding as well as the coagulation continued between 50° and 70°C and appeared to be complete above 70°C.

The principal heme pigment of cooked meat has been identified as a denatured nicotinamide hemichrome produced from myoglobin (American Meat Institute Foundation, 1960). Myoglobin was considered by Hamm to

be coagulated at 65°C. However, Bernofsky et al. (1959) noted that considerable denaturation occurred below 65°C in myoglobin of beef muscle. Enzymatic activity as well as heat was thought by Hamm (1966) to contribute to myoglobin denaturation at temperatures below 65°C.

CHAPTER III

PROCEDURE

I. STUDY PLAN

The effect of heat on myoglobin content of pectoralis major and thigh muscle pieces from nine turkey toms was studied. Right and left pectoralis major and thigh muscles were each divided into a total of four pieces, and one of each group was used as a raw sample. The three remaining pieces of each muscle were heated to 35°, 50°, and 65°C, respectively, according to the plans for treatments (Cochran et al., 1964) indicated in Tables 1 and 2. All the samples at each cooking period were from the same bird and the same muscle. Each treatment was replicated nine times for the pectoralis major and nine times for the thigh muscles.

An analysis of variance was used to assess the data. Duncan's multiple range test was used to locate significant differences among means of myoglobin concentration on a wet and a nonfat dry weight basis as well as moisture and fat content, with respect to heat treatment within each replication (Steele et al., 1960). A Student's paired t comparison was used to determine significant differences in myoglobin concentration between the thigh and pectoralis major.

II. MEAT SOURCE

Nine frozen U. S. Grade A turkey toms of similar brand, weight class (20 lb. 2 oz. - 21 lb. 9 oz.), and code number were purchased at

TABLE 1. Plan for treatment of pectoralis major pieces[a]

	Right		Left	
	Anterior	Posterior	Anterior	Posterior
Cooking Period	°C	°C	°C	°C
I	35	65	Raw	50
II	65	35	50	Raw
III	Raw	50	35	65
IV	50	Raw	65	35
V	35	Raw	50	65
VI	65	50	Raw	35
VII	Raw	35	65	50
VIII	50	65	35	Raw
IX	35	50	65	Raw

[a]Plan 13.22 Cockran et al. (1964).

TABLE 2. Plan for treatment of thigh pieces[a]

Cooking Period	Right		Left	
	Inner °C	Outer °C	Inner °C	Outer °C
I	35	65	Raw	50
II	65	35	50	Raw
III	Raw	50	35	65
IV	50	Raw	65	35
V	35	Raw	50	65
VI	65	50	Raw	35
VII	Raw	35	65	50
VIII	50	65	35	Raw
IX	35	50	65	Raw

[a]Plan 13.22 Cochran et al. (1964).

the same time from a local meat and poultry packing plant and stored at -18°C (0°F) in an institutional size freezer until needed for sampling.

III. SAMPLE PREPARATION

Prior to sampling, the Cry-O-Vac packaged birds were defrosted one at a time on a rack for 24 hours at 23°C (74°F) and for 20 hours at 3°C (37.4°F). The pectoralis major and the thigh then were removed from the defrosted birds. Paired pectoralis major muscles of each turkey were divided laterally into a total of four pieces, and paired thighs were divided longitudinally, also into four pieces.

Turkey pieces were cooked on a wire mesh rack standing 1 1/2 inches from the bottom of a shallow broiling pan. Internal temperatures were determined by thermocouples attached to a multipoint temperature recorder. Samples, with an initial internal temperature of 4°C (40 ± 2°F), were cooked to 35°, 50°, and 65°C (95°, 122°, and 149°F) in a 160 ± 6°C (320 ± 10°F) preheated oven of an electric range. The time required to reach the end points was recorded as well as the maximum temperature reached following the removal of the meat from the oven. The increase in temperature was similar for the pectoralis major and the thigh with an average rise of 6°C for the 35°C samples, 3°C for the 50°C samples, and 2°C for the 65°C samples.

The samples were cooled to temperatures between 25° and 40°C, cut into approximately one inch segments to facilitate grinding, packaged, labeled, and stored at -18°C (0°F) until ready for use. In

preparation for extraction of myoglobin, the frozen samples were ground twice, packaged, labeled, and returned to storage at -18°C (0°F).

IV. MYOGLOBIN DETERMINATION

The method for the quantitative determination of myoglobin spectrophotometrically was that adopted by Ginger et al. (1954) and later used by Froning et al. (1968). Twenty g. of twice ground meat was mixed with 20 ml. distilled, demineralized water and allowed to remain 24 hours at 3-5°C. The slurry was centrifuged in an International Model U centrifuge at 2000 x G for 15 minutes. The pH of the supernatant fluid containing the myoglobin was adjusted to pH 7 with a phosphate buffer of pH 8.35. Saturated basic lead acetate equal to 0.25 the volume of the supernatant fluid was added to precipitate proteins other than myoglobin and hemoglobin. The precipitate was removed by centrifugation (2000 x G for 15 minutes). Mono- and di-basic potassium phosphate (2.05 and 2.61 g., respectively) were added to a 10 ml. aliquot of the supernatant fluid to bring the phosphate concentration to 3 M and the pH to 6.6. This step precipitated the hemoglobin and left the myoglobin in solution. The precipitate was removed by centrifugation at 2000 x G for 15 minutes, and the supernatant fluid was filtered through Whatman #1 filter paper. Potassium ferricyanide (2 mg.) was added to make the final ferricyanide concentration 0.6 mM./l., and sodium cyanide (0.4 mg.) was added to provide a final cyanide concentration of 0.8 mM./l. The absorbance of the resultant cyanmetmyoglobin was read on a Bausch and Lomb Spectronic 20 at a

wavelength of 540 mμ. Total molar concentration of the pigment was determined using Drabkin's (1950) molar extinction coefficient of 11,300 for cyanmetmyoglobin at a wavelength of 540 mμ. Molar concentration was converted to mg. of pigment/g. of tissue by the formula

$$\frac{O.D.}{11,300}\left[\frac{17,000 \times \text{volume of extract in l.} \times 1,000}{\text{g. sample}}\right]$$

where $\frac{O.D.}{11,300}$ equals molar concentration of pigment, O.D. equals absorbance at 540 mμ, 11,300 equals molar extinction coefficient of cyanmetmyoglobin at 540 mμ, and 17,000 equals the molecular weight of myoglobin. The equation was multiplied by 1,000 to convert g. pigment to mg. (Fleming et al., 1960; Rickansrud et al., 1967).

V. MOISTURE AND FAT DETERMINATION

Moisture and fat content was determined in duplicate for each sample and the average per cent fat free dry weight was calculated. Approximately five g. of the ground sample was placed in a weighed Whatman 22 x 80 mm. single thickness extraction shell. The samples were dried in a warm oven (65°C) prior to drying for 16 hours in a vacuum oven at 65°C. The samples were cooled one hour in a dessicator, weighed, and per cent moisture was calculated. A Goldfisch apparatus was used for fat extraction. The samples were extracted for four hours with petroleum ether then allowed to stand at room temperature until ether fumes were no longer detectable. Shells containing the extracted samples were then dried at 65°C in a vacuum oven to two hours, cooled for one hour in a dessicator, weighed, and per cent fat was calculated (Association of Official Agricultural Chemists, 1965).

CHAPTER IV

RESULTS AND DISCUSSION

I. MYOGLOBIN CONCENTRATION AS AFFECTED BY HEAT

The concentration of myoglobin as expressed on the basis of wet and nonfat dry tissue is presented in Table 3. Myoglobin concentration of the pectoralis major tended to decrease with increasing internal temperature when expressed as mg./g. of wet or of nonfat dry tissue. However, this decrease was not significant. Since initial concentrations of myoglobin in the pectoralis major muscle were small, the method may not have been sensitive enough to detect small decreases in concentration.

Myoglobin concentration of the thigh decreased with increasing internal temperature ($P < 0.005$) when expressed as mg./g. of wet and of nonfat dry tissue. Raw and 35°C samples were similar in myoglobin concentration when expressed either on a wet or nonfat dry weight basis (Table 4). When expressed on the wet basis, concentration of 35° and 50°C samples was similar, and concentration of 50° and 65°C samples also was similar. However, on the nonfat dry weight basis, 35°C samples had a higher ($P < 0.005$) concentration of myoglobin than that cooked to 50°C. Thus, myoglobin concentration did not decrease until samples were cooked to 50°C, and some myoglobin was still present at 65°C. In intact tissue such as this, heat is not the only factor affecting denaturation, as indicated by Bernofsky et al. (1959).

TABLE 3. Myoglobin concentration of pectoralis major and thigh as affected by heat

Muscle	mg./g. of Tissue				Significance of F Values
	Raw	35°C	50°C	65°C	
Pectoralis Major					
Wet	0.57	0.57	0.41	0.26	NS
Nonfat Dry	2.16	2.00	1.40	0.83	NS
Thigh					
Wet	2.21	1.94	1.33	0.84	0.005
Nonfat Dry	9.98	8.17	5.23	3.05	0.005

TABLE 4. Significance of Duncan's multiple range values for myoglobin concentration of pectoralis major and thigh[a]

Muscle	Treatment °C				Significance
Pectoralis Major					
Wet	65	50	35	Raw	NS
Nonfat Dry	65	50	35	Raw	NS
Thigh					
Wet	65	50	35	Raw	0.005
Nonfat Dry	65	50	35	Raw	0.005

[a]Treatments within a muscle and a method differ from each other when not underscored by a continuous line.

Although at temperatures of 65°C and below, denaturation of intact beef tissue was considerable, denaturation of the pigment in pure solution at these temperatures was negligible. In intact muscle tissue enzymatic activity and pH changes during heating might be factors affecting myoglobin denaturation.

Myoglobin concentrations expressed on either a wet or a nonfat dry weight basis were greater for the thigh ($P < 0.001$) than the pectoralis major as indicated by a Student's paired t comparison. A higher degree of muscle activity in the thigh than in the pectoralis major might have caused a greater need for oxygen and ultimately a more efficient oxygen storage mechanism and more myoglobin (American Meat Institute Foundation, 1960).

The values for myoglobin concentration of both the pectoralis major and the thigh muscles obtained in this study were higher than those reported by Froning et al. (1968) in the only study of turkey myoglobin concentration found in the literature. In the Froning study myoglobin concentration increased as birds increased in age from 14 to 28 weeks. Although exact age of the birds used in this study was not known, they were mature birds and possibly older than 28 weeks. Another possible explanation for differences is based on the molar extinction coefficient assumed. Since Froning followed the method of Ginger et al. (1954), he probably used the molar extinction coefficient of cyanmethemoglobin, 11,500. The molar extinction of cyanmetmyoglobin, 11,300, as reported by Drabkin (1950), Fleming et al. (1960), and Rickansrud et al. (1967), was used in this study. Using this smaller value might have

contributed to the different values. Another difference between this and the Froning study involved the molecular weight of myoglobin used in converting the concentration from mM./l. to mg./g. of wet tissue. Although the Nebraska workers did not report the molecular weight used in the conversion, it is probable that it was 16,500 as used by Ginger et al. (1954). A molecular weight of 17,000 was employed in this study which might account for the somewhat higher values for myoglobin concentration.

II. MOISTURE AND FAT CONTENT

Per cent moisture and fat in the pectoralis major tended to decrease as end point temperature increased (Table 5). Moisture in each treatment was smaller ($P < 0.005$) than in preceding treatments except between 35° and 50°C (Table 6). Fat also decreased as temperature increased ($P < 0.05$) with the raw and 35°C samples being similar, and the 35°, 50°, and 65°C samples being similar. Differences in fat were indicated between raw and 50°C, and raw and 65°C.

Moisture content of the thigh also decreased as temperature increased ($P < 0.005$), and the values obtained for each treatment were different from each other. However, fat content of thigh pieces was similar and unrelated to end point temperature (Table 5). Moisture content tended to be higher in the thigh than the pectoralis major for the raw and 35°C samples, but not the 50° and 65°C samples. Fat content tended to be higher in the thigh than in the pectoralis major for all treatments.

TABLE 5. Moisture and fat content of pectoralis major and thigh as affected by heat

Muscle	Per Cent				Significance of F Values
	Raw	35°C	50°C	65°C	
Pectoralis Major					
Moisture	72.94	70.95	70.14	68.63	0.005
Fat	0.50	0.45	0.25	0.27	0.05
Thigh					
Moisture	74.40	72.13	70.60	68.30	0.005
Fat	3.40	3.40	3.81	3.58	NS

TABLE 6. Significance of Duncan's multiple range values for moisture and fat content of pectoralis major and thigh[a]

Muscle	Treatment °C				Significance
Pectoralis Major					
Moisture	65	50	35	Raw	0.005
Fat	50	65	35	Raw	0.05
Thigh					
Moisture	65	50	35	Raw	0.005
Fat	35	Raw	65	50	NS

[a]Treatments within a muscle and a method differ from each other when not underscored by a continuous line.

III. RECOMMENDATIONS

Although the method used in this study provided an effective means for quantitative determination of myoglobin spectrophotometrically, certain problems arose that may be attributed to sample size. In the method described by Froning et al. (1968) that was used in this study, a 10 g. sample was extracted with 10 ml. distilled water. Preliminary work indicated that the quantity of extract was too small to accurately adjust the pH, thus the quantity of both meat and water was doubled. This facilitated pH adjustment, but determinations following the lead acetate precipitation were made on a 10 ml. aliquot. Even though this sample quantity was more workable than at the beginning of the procedure, a larger quantity would have permitted the increase in amounts of reagents needed and a smaller possible error in weighing techniques.

Filtration after addition of the phosphates was slow. If samples were allowed to stand for extended periods of time, they tended to become cloudy, and a possible experimental error was introduced in optical density readings. Cyanides need to be added immediately and optical density read as soon as possible to minimize experimental error.

CHAPTER V

SUMMARY

The effect of heat on myoglobin concentration of pectoralis major and thigh muscles of nine turkey toms was studied. Heat treatments included end point temperatures of 35°, 50°, and 65°C.

Myoglobin concentration of the pectoralis major as expressed on a wet and a nonfat dry basis did not change with increasing temperature. Initial myoglobin concentration in the pectoralis major was small, and the method might not have been sensitive enough to detect small decreases in concentration. Myoglobin concentration in the thigh on both a wet and a nonfat dry weight basis decreased as temperature increased ($P < 0.005$). The concentration of the pigment did not decrease until the samples were cooked to 50°C, and some myoglobin was still present at 65°C. Myoglobin concentration of the thigh was larger than that of the pectoralis major on both a wet and a nonfat dry basis ($P < 0.001$).

Moisture decreased in both the pectoralis major and the thigh as temperature increased ($P < 0.005$) and tended to be higher in the thigh than in the pectoralis major for the raw and 35°C samples, but not the 50° and 65°C samples. Fat also decreased in the pectoralis major as temperature increased ($P < 0.05$) but, although higher in the thigh than in the pectoralis major, was unrelated to temperature.

REFERENCES

REFERENCES

American Meat Institute Foundation. 1960. "The Science of Meat and Meat Products," pp. 10-151. W. H. Freeman and Co., San Francisco.

Association of Official Agricultural Chemists. 1965. "Official Methods of Analysis." Association of Official Agricultural Chemists, Washington.

Bernofsky, C., Fox, J. B., Jr., and Schweigert, B. S. 1959. Biochemistry of myoglobin. VII The effect of cooking on myoglobin in beef muscle. Food Res. 24, 339-343.

Cochran, W. G., and Cox, G. M. 1964. "Experimental Designs," p. 537. John Wiley and Sons, Inc., New York.

Conn, E. E., and Stumpf, P. K. 1966. Amino acids and proteins. In "Outlines of Biochemistry," pp. 66-93. John Wiley and Sons, Inc., New York.

Crandall, M. W., and Drabkin, D. L. 1946. Cytochrome c in regenerating rat liver and its relation to other pigments. J. Biol. Chem. 166, 653-668.

Drabkin, D. L. 1950. The distribution of the chromoproteins, hemoglobin, myoglobin, and cytochrome c, in the tissues of different species, and the relationship of the total content of each to body mass. J. Biol. Chem. 182, 317-333.

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, 51-65.

Fleming, H. B., Blumer, T. N., and Craig, H. B. 1960. Quantitative estimations of myoglobin and hemoglobin in beef muscle extracts. J. Animal Sci. 19, 1164-1171.

Fox, S. W., and Foster, J. F. 1957. Denaturation of proteins. In "Introduction to Protein Chemistry," pp. 304-326. John Wiley and Sons, Inc., New York.

Froning, G. W., Daddario, J., and Hartung, T. W. 1968. Color and myoglobin concentration in turkey meat as affected by age, sex, and strain. Poultry Sci. 47, 1827-1835.

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, 1037-1040.

Hamm, R. 1966. Heating of muscle systems. In "The Physiology and Biochemistry of Muscle as a Food," ed. Briskey, E. J., Cassens, R. G., and Trautman, J. C., pp. 363-386. The University of Wisconsin Press, Madison.

Hamm, R., and Deatherage, F. E. 1960. Changes in hydration solubility and charges of muscle proteins during heating of meat. Food Res. 25, 587-610.

Joly, M. 1965. "A Physico-chemical Approach to the Denaturation of Protein." Academic Press, New York.

Kendrew, J. C. 1963. Myoglobin and the structure of proteins. Science 139, 1259-1266.

Lawrie, R. A. 1950. Some observations on factors affecting myoglobin concentration in muscle. J. Agr. Sci. 40, 356-366.

Lawrie, R. A. 1966. "Meat Science," pp. 66-115. Pergamon Press, London.

Morgan, V. E. 1936. Studies on myoglobin. I The solubility of myoglobin in concentrated ammonium sulfate solutions. J. Biol. Chem. 112, 557-565.

Rickansrud, D. A., and Hendrickson, R. L. 1967. Total pigments and myoglobin concentration in four bovine muscles. J. Food Sci. 32, 57-61.

Steele, R. G. D., and Torrie, J. H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., New York.

Tanford, C. 1968. Protein denaturation. In "Advances in Protein Chemistry," p. 23, ed. Anfinsen, C. B., Jr., Anson, M. L., Edsall, J. T., and Richards, F. M., pp. 121-282. Academic Press, New York.

Watts, B. M. 1954. Oxidative rancidity and discoloration in meat. Adv. in Food Res. 5, 1-52.

APPENDIX

TABLE 7. Myoglobin concentration of pectoralis major as affected by heat

Replication	mg./g. Wet Tissue				mg./g. Nonfat Dry Tissue			
	Raw	35°C	50°C	65°C	Raw	35°C	50°C	65°C
I	0.30	0.23	0.30	0.30	1.17	0.82	1.04	0.92
II	0.75	0.60	0.30	0.23	2.78	2.15	1.01	0.75
III	1.50	1.43	1.05	0.15	5.60	4.77	3.44	0.45
IV	0.15	0.30	0.53	0.22	0.55	1.02	1.76	0.73
V	0.90	0.98	0.30	0.53	3.41	3.39	1.02	1.69
VI	0.23	0.53	0.38	0.23	0.89	1.94	1.30	0.74
VII	0.08	0.15	0.23	0.15	0.29	0.51	0.76	0.49
VIII	0.68	0.53	0.38	0.30	2.50	1.83	1.28	0.95
IX	0.60	0.45	0.30	0.23	2.28	1.60	1.00	0.75
Averages	0.57	0.57	0.41	0.26	2.16	2.00	1.40	0.83

TABLE 8. Myoglobin concentration of thigh as affected by heat

Replication	mg./g. Wet Tissue				mg./g. Nonfat Dry Tissue			
	Raw	35°C	50°C	65°C	Raw	35°C	50°C	65°C
I	1.66	1.50	0.68	0.30	7.55	6.19	2.49	0.93
II	2.41	2.41	1.58	1.50	10.53	9.84	6.64	5.63
III	3.01	2.63	1.35	0.60	13.61	11.45	5.15	2.08
IV	2.56	2.18	2.56	1.05	11.20	8.85	10.38	4.04
V	1.80	2.18	1.80	0.83	7.87	9.11	7.19	2.89
VI	2.11	1.35	0.90	0.75	10.09	5.41	3.42	2.73
VII	2.03	1.13	1.05	0.83	9.45	4.47	3.50	3.07
VIII	2.18	1.96	0.90	0.98	9.62	8.70	3.67	3.29
IX	2.18	2.18	1.20	0.75	9.92	9.53	4.63	2.83
Averages	2.21	1.94	1.33	0.84	9.98	8.17	5.23	3.05

TABLE 9. Per cent moisture of pectoralis major and thigh as affected by heat

	Pectoralis Major				Thigh			
Replication	Raw	35°C	50°C	65°C	Raw	35°C	50°C	65°C
I	74.08	71.99	71.03	67.40	74.46	72.22	69.92	65.36
II	72.60	71.52	70.32	69.12	74.78	72.95	70.94	69.72
III	72.84	70.10	69.56	67.37	74.08	73.39	70.54	67.60
IV	72.29	70.44	69.92	69.76	73.64	71.78	71.56	69.16
V	73.30	70.83	70.41	68.55	75.24	73.38	70.08	68.99
VI	73.37	72.11	70.34	68.70	74.72	72.36	71.04	69.77
VII	72.92	70.12	69.80	69.28	73.89	70.98	70.05	67.93
VIII	71.98	69.98	69.97	68.35	73.85	72.24	70.56	66.79
IX	73.10	71.54	69.96	69.22	74.94	69.90	70.78	69.40
Averages	72.94	70.95	70.14	68.63	74.40	72.13	70.60	68.30

TABLE 10. Per cent fat of pectoralis major and thigh as affected by heat

	Pectoralis Major				Thigh			
Replication	Raw	35°C	50°C	65°C	Raw	35°C	50°C	65°C
I	0.29	0.22	0.18	0.32	3.53	3.54	2.80	2.46
II	0.48	0.58	0.24	0.33	2.32	2.55	5.26	3.66
III	0.38	0.00	0.00	0.00	3.79	3.62	3.26	3.64
IV	0.46	0.35	0.08	0.34	3.50	3.58	3.77	4.84
V	0.36	0.31	0.16	0.07	1.88	2.69	4.87	2.34
VI	0.77	0.62	0.48	0.40	4.35	2.65	2.65	2.76
VII	0.31	0.52	0.29	0.22	4.65	3.74	3.50	5.01
VIII	0.87	1.08	0.54	0.36	3.50	5.24	4.93	3.44
IX	0.60	0.44	0.29	0.43	3.08	3.00	3.30	4.15
Averages	0.50	0.45	0.25	0.27	3.40	3.40	3.81	3.58

TABLE 11. Analysis of variance of myoglobin concentration and moisture and fat content of pectoralis major

	Myoglobin			
	Wet	Nonfat Dry	Moisture	Fat
Degrees of Freedom				
Treatment	3	3	3	3
Error	32	32	32	32
Total	35	35	35	35
Sum of Squares				
Treatment	0.60	10.01	86.82	0.44
Error	3.50	43.34	15.81	1.48
Total	4.10	53.35	102.63	1.92
Mean Square				
Treatment	0.20	3.33	28.94	0.14
Error	0.10	1.35	0.49	0.04
F Value	2.00	2.46	59.06	3.50
Sig. of F	NS	NS	0.005	0.05

TABLE 12. Rp values for Duncan's multiple range of myoglobin concentration and moisture and fat content of pectoralis major

	Myoglobin			
p	Wet	Nonfat Dry	Moisture	Fat
2	--	--	1.00	0.21
3	--	--	1.04	0.22
4	—	—	1.07	0.22

TABLE 13. Analysis of variance of myoglobin concentration and moisture and fat content of thigh

	Myoglobin			
	Wet	Nonfat Dry	Moisture	Fat
Degrees of Freedom				
Treatment	3	3	3	3
Error	32	32	32	32
Total	35	35	35	35
Sum of Squares				
Treatment	10.25	255.18	177.81	1.05
Error	6.93	130.30	33.26	26.74
Total	17.18	385.48	211.07	27.79
Mean Square				
Treatment	3.41	85.06	59.27	0.35
Error	0.21	4.07	1.04	0.83
F Value	16.23	20.89	56.99	0.42
Sig. of F	0.005	0.005	0.005	NS

TABLE 14. Rp values for Duncan's multiple range of myoglobin concentration and moisture and fat content of thigh

	Myoglobin			
p	Wet	Nonfat Dry	Moisture	Fat
2	0.66	2.88	1.46	--
3	0.68	3.00	1.51	--
4	0.70	3.07	1.55	--

VITA

Janice Gail Sandefer was born in Martin, Tennessee, on August 17, 1947. She attended elementary schools in that city and was graduated from Martin High School in May, 1965. That summer she entered The University of Tennessee at Martin and began work on a Bachelor of Science degree in Home Economics Education, which was completed in December, 1968. While at The University of Tennessee at Martin, she became a charter member of Phi Beta Alpha, a local home economics honorary, and was elected to Who's Who in American Colleges and Universities.

She entered the Graduate School at The University of Tennessee, Knoxville, in January, 1969, began work on the Master of Science degree with a major in Food Science, and completed degree requirements in March, 1970. She is a member of American Home Economics Association, the Institute of Food Technology, Omicron Nu, and Alpha Delta Pi.