Inhibition of Pinking in Cooked, Uncured Turkey Rolls Through the Binding of Non-Pinking Ligands to Muscle Pigments

by

Stephen J. Schwarz

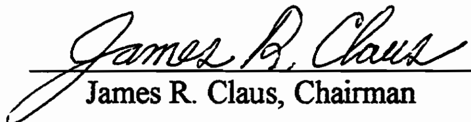
Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

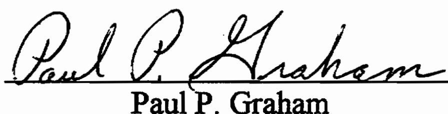
MASTER OF SCIENCE

in

Food Science and Technology

APPROVED:


James R. Claus, Chairman


Paul P. Graham

Norman G. Marriott

May 17, 1996

Blacksburg, Virginia

Key words: Turkey, Pinking Inhibition, Ligands, Pigments

c.2

LD
5655
V855
1996
S394
c.2

INHIBITION OF PINKING IN COOKED, UNCURED TURKEY THROUGH THE BINDING OF NON-PINKING LIGANDS TO MUSCLE PIGMENTS

by

Stephen J. Schwarz

Chairman: James R. Claus

Department of Food Science and Technology

(ABSTRACT)

The pink color defect in cooked, uncured turkey is a sporadic problem which can result in consumer dissatisfaction. Nicotinamide hemochrome may be one of the major pigments responsible for this defect. Reflectance (400-700 nm) methodology was developed to reliably and easily quantify (%R 537 nm/%R 553 nm; r = 0.993) the presence of nicotinamide hemochrome.

Fourteen ligands were tested in a ground turkey system to determine their ability to reduce pinking in control samples and in the presence of pinking agents (1.0% nicotinamide or 150 ppm sodium nitrite). Trans 1,2-diaminocyclohexane-N,N,N',N' tetraacetic acid monohydrate (CDTA); diethylenetriamine pentaacetic acid (DTPA), ethylenedinitrilo-tetraacetic acid disodium salt (EDTA), and calcium reduced non-fat dry milk (NFDM) were the most effective at reducing pinking with and without pinking agents.

These four ligands were evaluated in intact turkey breasts with and without added pinking agents (1.0% nicotinamide or 20 ppm, 100 ppm sodium nitrite). Ligands were evaluated at various levels (50, 100, 200 ppm; except NFDM 1.0% or 2.0%), over three storage times (1, 14, 21 days), and after two minutes of exposure to light and air. When

ligands were evaluated without pinking agents, samples were generally lighter and less pink than controls. In the presence of added pinking agents, the ligands were more successful at reducing nicotinamide pink than nitrite pink. As storage time increased, samples became more pink and the addition of ligands was only successful in delaying this affect. One of the most effective ligands, DTPA, reduced the sample CIE a* value 31.7% when tested alone and 30.8% in the presence of nicotinamide. NICHEM was also effectively reduced by DTPA. In general, 50 ppm of added ligand was sufficient to produce a significant reduction in pinking.

ACKNOWLEDGEMENTS

It is with sincere gratitude and appreciation that I acknowledge the individuals who have made this accomplishment possible. I have never seen an individual with the drive, commitment, and attention to detail that Dr. James R. Claus exhibited time and again. It was his guidance, dedication, and fine example that kept me moving forward even when motivation was low. Dr. Claus has instilled a strong commitment to excellence in me for which I will always be grateful.

Thanks is also expressed to my committee members Drs. Paul Graham and Norman Marriott for their time and review. Special thanks goes to Dr. Hengjian Wang who never failed to take time out of his busy schedule to assist me in solving a world of statistical problems I didn't even comprehend. John Chandler and Brent Green were also invaluable to the success of this project. John was always willing to set aside time to help me work out mechanical problems when they arose while Brent logged in late night hours in the meats lab and offered unconditional friendship. Both individuals showed me the value of a strong spiritual relationship with God, without whom none of this would have been possible.

My love and respect goes to my wife Rita who stood by me, put up with me, and encouraged me at the appropriate times. On May 8, 1996 she brought me the gift of our son, Tobias Joseph. I never knew how much joy a child could bring.

Because of the strong academic education and research experience that I received from the Food Science and Technology Department at Virginia Tech, I have no doubt that my career and character will continue to grow from that foundation.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES AND FIGURES	ix
1.0 Introduction	1
2.0 Literature Review	8
2.1 Native Muscle Pigments	8
2.1.1 Hemoglobin	8
2.1.2 Myoglobin	11
2.1.3 Cytochrome c	12
2.2 Heme Pigment Structure and Chemical States	14
2.2.1 Heme pigment structure	14
2.2.2 Iron oxidation states	15
2.2.3 Nature of the heme-ligand bond	16
2.3 Ligands that Bind Heme Pigments	20
2.3.1 Ferrous and ferric derivatives of native myoglobin and cytochrome c	20
2.3.2 Ferrous and ferric derivatives of denatured myoglobin and cytochrome c	25
2.3.3 Spectra of native and denatured heme complexes	28
2.4 Factors Affecting Ligand Binding and Ligand Availability	31
2.4.1 Pigment denaturation	31
2.4.2 pH, salt and phosphates	36
2.4.3 Oxidation-reduction potential	38
2.5 Chemical and Electronic Basis for Visually Perceived Colors	39
2.5.1 Electronic nature of heme and globin	39
2.5.2 Effect of bound ligand on color	39

2.6 Pinking Defect.... 40
2.6.1 Location and type of products affected.... 40
2.6.2 Principle pigments involved.... 41
2.6.3 Effect of end-point cooking temperature.... 42
2.6.4 Effect of nitrogenous contamination.... 43
2.6.5 Effect of storage time and temperature.... 46
2.6.6 Effect of oxidation-reduction potential.... 46

2.7 Strategies for the Prevention of Pinking.... 47

2.8 References.... 49

3.0 Nicotinamide Hemochrome Quantification Methodology.... 55
3.1 Abstract.... 55

3.2 Introduction.... 55

3.3 Materials and Methods.... 57
3.3.1 Preparation of Turkey.... 57
3.3.2 Sample Treatments and Analysis.... 58
3.3.3 Manipulation of Reflectance Data.... 59
3.3.4 Statistical Analysis.... 59

3.4 Results and Discussion.... 59

3.5 Conclusions.... 62

3.6 References.... 63

4.0 Preliminary Screening of Ligands to Inhibit Pinking in Ground, Cooked, Uncured Turkey.... 69
4.1 Abstract.... 69

4.2 Introduction.... 69

4.3 Materials and Methods.... 71
4.3.1 Preparation of Meat Mix.... 71
4.3.2 Processing of Meat Mix.... 73
4.3.3 Characterization of Color.... 74
4.3.4 Statistical Analysis.... 74

4.4 Results and Discussion.... 75
4.4.1 Effect of Ligands Without Added Pinking Agents.... 75

4.4.2 Effect of Ligands With Sodium Nitrite Added as a Pinking Agent........ 78
4.4.3 Effect of Ligands With Nicotinamide Added as a Pinking Agent........ 79
4.3.4 Overall Effectiveness of Ligands........ 80

4.5 Conclusions........ 80

4.6 References........ 81

5.0 Inhibition of Pinking in Cooked, Uncured Turkey Rolls Through Binding of Non-Pining Ligands to Muscle Pigments........ 86
5.1 Abstract........ 86

5.2 Introduction........ 86

5.3 Materials and Methods........ 89
5.3.1 Raw Material Selection and Characterization........ 89
5.3.2 Preparation of Treatment Solutions........ 90
5.3.3 Preparation of Turkey Rolls........ 90
5.3.4 Evaluation of Cooked Breasts........ 91
5.3.5 Statistical Analysis........ 93

5.4 Results and Discussion........ 93
5.4.1 Effect of Each Ligand on Sample pH........ 93
5.4.2 Effect of Each Ligand on Cooking Loss........ 94
5.4.3 Effect of Ligand Level of Each Ligand on Dependent Variables........ 95
5.4.4 Effect of Storage Time on Dependent Variables for Each Ligand........ 99
5.4.5 Effect of Each ligand on Fading Time........ 103
5.4.6 Effects of Ligands Over All Levels, Storage Times, and Fading Times........ 105

5.5 Conclusions........ 106

5.6 References........ 107

6.0 Summary and Conclusions........ 120

7.0 Appendix........ 122
7.1 Myoglobin Concentration........ 122
7.2 Effect of Each Ligand on Native Myoglobin Derivatives........ 122
7.3 References........ 131

Vita........ 132

Listing of Tables and Figures

Page

Chapter 2: Literature Review

Table 1. Species and muscle differences in myoglobin and cytochrome c level 10
Figure 1. Structure of heme group 15
Figure 2. Electron distribution of iron 16
Figure 3. Bonding geometry for the MbO_2 complex 17
Figure 4. Energy level diagrams for 3d electrons 19
Figure 5. Energy level diagram for MbO_2 complex 21
Table 2. Absorbance peaks indicative of common heme pigment derivatives 29

Chapter 3: Nicotinamide Hemochrome Quantification

Table 1. Correlation coefficients (r) for the ratios of 533-539 nm and 552-558 nm wavelength ranges for nicotinamide and sodium nitrite containing turkey samples 64
Figure 1. Reflectance verses wavelength for control samples treated with nothing, light, hydrogen peroxide, potassium ferricyanide, and sodium dithionite 65
Figure 2. Reflectance verses wavelength for 2.0 % nicotinamide samples treated with nothing, light, hydrogen peroxide, potassium ferricyanide, and sodium dithionite 66
Figure 3. Reflectance verses wavelength for 75 ppm sodium nitrite samples treated with nothing, light, hydrogen peroxide, potassium ferricyanide, and sodium dithionite 67
Figure 4. Control and nicotinamide level reflectance graphs 68

Chapter 4: Ligand Screening

Table 1. Ligand screening treatment specifications 72
Table 2. Ligand effects on instrumental color and pigment measurements in the absence of added pinking agents 83
Table 3. Ligand effects on instrumental color and pigment measurements in the presence of added sodium nitrite (150 ppm) 84
Table 4. Treatment effects on instrumental color and pigment measurements in the presence of added nicotinamide (1.0%) 85

Chapter 5: Comprehensive Testing

Table 1. Effect of ligand and ligand level on color and nicotinamide hemochrome for cooked turkey breasts without pinking agents... 109
Table 2. Effect of ligand and ligand level on color and nicotinamide hemochrome for cooked turkey breasts with nicotinamide as a pinking agent........ 110
Table 3. Effect of ligand and ligand level on color and hemochrome values for cooked turkey breasts with sodium nitrite........ 111
Table 4. Mean sensory scores for each ligand and ligand level with and without a pinking agent........ 112
Table 5. Effect of ligand and storage time on color and nicotinamide hemochrome for cooked turkey breasts without a pinking agent... 113
Table 6. Effect of ligand and storage time on color and nicotinamide hemochrome for cooked turkey breasts with nicotinamide as a pinking agent........ 114
Table 7. Effect of ligand and storage time on color and hemochrome values for cooked turkey breasts with sodium nitrite........ 115
Table 8. Effect of ligand and fading time on color and nicotinamide hemochrome for cooked turkey breasts without a pinking agent.... 116
Table 9. Effect of ligand and fading time on color and nicotinamide hemochrome for cooked turkey breasts with nicotinamide as a pinking agent........ 117
Table 10. Effect of ligand and fading time on color and hemochrome values for cooked turkey breasts with sodium nitrite........ 118
Table 11. Overall effect of each ligand on color and hemochrome values for cooked turkey breasts with and without a pinking agent............ 119

Chapter 7: Appendix

Table 1. Effect of ligand and ligand level on myoglobin derivatives for cooked turkey breasts without pinking agents........ 123
Table 2. Effect of ligand and ligand level on myoglobin derivatives for cooked turkey breasts with nicotinamide as a pinking agent......... 124
Table 3. Effect of ligand and ligand level on myoglobin derivatives for cooked turkey breasts with sodium nitrite as a pinking agent......... 125
Table 4. Effect of ligand and storage time on myoglobin derivatives for cooked turkey breasts without pinking agents........ 126
Table 5. Effect of ligand and storage time on myoglobin derivatives for cooked turkey breasts with nicotinamide as a pinking agent.......... 127
Table 6. Effect of ligand and storage time on myoglobin derivatives for cooked turkey breasts with sodium nitrite as a pinking agent......... 128
Table 7. Effect of ligand and fading time on myoglobin derivatives for cooked turkey breasts without pinking agents........ 129

Table 8. Effect of ligand and fading time on myoglobin derivatives for cooked turkey breasts with nicotinamide as a pinking agent............ 130
Table 9. Effect of ligand and fading time on myoglobin derivatives for cooked turkey breasts with sodium nitrite as a pinking agent............ 131

Chapter 1

Introduction

There are many characteristics that consumers evaluate when making the decision to purchase meats. Perhaps the most utilized characteristic is color because it is the easiest quality trait to evaluate. Consumers have certain color and appearance expectations that are dependent upon meat product type. For example, cured products are expected to have a uniform, stable pink color while cooked beef steaks are acceptable over the range from red to brown. For food scientists and the producers of meat, it is crucial that the basis of meat color be understood in order to produce products that meet or exceed consumer expectations.

The three native pigments that are primarily responsible for meat color are the heme pigments hemoglobin, myoglobin and cytochrome c. The structure of native pigments relates to their function and how each interacts with the meat environment. Heme pigments consist of a protein (globin) and non-protein (heme ring) subunit. The heme ring, which is partially enclosed by the globin, is the reactive portion of native pigments. The oxidation state of the heme iron and the type of ligand bound to the iron determines the color and reactivity of the pigment (Livingston and Brown, 1981). Environmental conditions (oxidation-reduction potential, temperature, pH, salt, phosphates) are subject to change and influence pigment oxidation state and therefore ligand binding.

A major problem that has plagued the poultry industry, and other meat industries to a lesser degree, is the pink color defect. The pinking defect, also known as pink spot and pink ring, manifests itself as a pink coloring of the fresh cut surface of cooked meat products (Cornforth et al., 1986) such as cooked, uncured turkey and pork rolls. A wide variety of causes have been suggested that lead to pinking. Ghorpade and Cornforth (1993) discovered undenatured oxymyoglobin and deoxymyoglobin to be the pigments responsible for the pink color in pork roasts cooked to 65°C. Pool (1956) found that carbon monoxide and nitric oxide generated by the flames of gas ovens can combine with myoglobin to form a pink color. Likewise, exhaust fumes from automobiles (carbon monoxide and nitric oxide) inhaled by poultry in route to processing facilities have been reported to cause a pink color (Froning et al., 1969b).

Nitrate and nitrite contamination during raising or processing of birds has been a widely used explanation for pinking. Nitrates and nitrites in the feed (Froning and Hartung, 1967) or drinking water (Froning et al., 1969a) of poultry may lead to a pink end product. Nitrate or nitrite contamination can also originate in the water utilized for carcass chilling (Mugler, et al., 1970) and processing equipment previously used for cured products. Shaw et al. (1992) found that ammonia contamination caused a pink defect in pork.

Cornforth et al. (1986) studied the pigment(s) responsible for the pink defect in turkey rolls. Commercial pink samples were analyzed and no residual nitrite or nitrosylhemochrome were found. Hornsey (1956) found nitrosylhemochrome to be extractable with an acetone-water solvent. The pink pigment in commercial samples

studied by Cornforth et al. (1986) could not be extracted in water, acetone or other solvents. From these findings they concluded that the pink defect was not due to nitrite contamination.

If nitrosylhemochrome is not the pigment responsible for the pink defect then other hemochromes must be responsible. It has been demonstrated that hemochromes can form by the interaction of other nitrogenous ligands such as specific amino acids (Akoyunoglou et al. 1963; Ahn and Maurer, 1990), proteins, and nicotinamide (Ahn and Maurer, 1990). Akoyunoglou et al. (1963) characterized hemochromes as having a typical dual absorption spectrum with maxima at 520-530 nm and 555-565 nm. Cornforth et al. (1986) found that cooked turkey with 2.0% added nicotinamide had a reflectance spectrum similar to a commercial pink turkey sample. Those two samples also exhibited the typical hemochrome absorption spectrum. Brown and Tappel (1957) observed a pink color in canned tuna and determined that the primary pigment was a mixed nicotinamide-denatured-globin hemochrome. The high nicotinamide content of turkey meat (0.083 mg/g; Richardson et al., 1980) compared to other species favors the formation of nicotinamide hemochromes. Denatured proteins, such as the globin portion of myoglobin, also may contribute to pinking through hemochrome formation.

While hemochromes are generally considered to form between a nitrogenous ligand and myoglobin, it has been shown that cytochrome c can also form hemochromes (Ahn and Maurer, 1990). The concentration of cytochrome c is about 0.013 mg/g in turkey light meat (Pikul et al., 1986), 0.015 mg/g in the *Longissimus dorsi (LD)* of beef (Drabkin, 1950) and 0.052 mg/g in the *LD* of pork (Girard et al, 1990). The

concentration of myoglobin is 0.58 mg/g in light turkey (Niewiarowicz et al., 1986), 3.42 mg/g in the *LD* of beef (Hunt and Hedrick, 1977), and 2.87 mg/g in the *LD* of pork (Topel et al., 1966). Because the myoglobin concentrations are considerably lower in turkey, the ratio of myoglobin to cytochrome c lower. It is possible, therefore, that cytochrome c hemochromes play a significant role in the pink defect of turkey.

Regardless of which native pigments or hemochromes are involved in the pinking defect, it is a problem that needs to be solved. The first step in eliminating pinking should be to control contamination of the meat by ligands such as nitrate, nitrite, ammonia, nitric oxide, carbon monoxide, etc. If the number of different ligands available to form heme complexes is lower, than fewer types of hemochromes will be formed.

Since hemochromes are formed when specific ligands bind the heme pigments, it may be possible to bind a ligand to the heme ring that does not cause a pink color. Dobson and Cornforth (1992) added non-fat dry milk (NFDM) to turkey rolls in an effort to reduce pinking. Turkey rolls with 3% added NFDM showed no visible pink discoloration and had a very low redness value. It should be noted that no pinking agents (e.g. sodium nitrite or nicotinamide) were added to produce a pink color. Since the pink defect occurs sporadically, they had no way of knowing if the elimination of pinking was due to chance or the effects of the NFDM. The mechanism of NFDM's action is unclear at this time. It may simply be that the casein micelles mask the pink color or that reactive protein side chains of the NFDM raised the oxidation-reduction potential. Another possibility is that the proteins in NFDM may denature at a lower temperature and bind with the heme ring to form a non pink complex. The NFDM may have also decreased

protein denaturation in the meat so that less amino acids were available to form hemochromes (Dobson and Cornforth, 1992).

Other ligands with pinking reduction potential may exist among compounds that were unsuccessfully tested as nitrite substitutes and among metal chelators used in medical therapy.

The remaining chapters of this thesis include: a literature review, three research chapters, and conclusions.

The objectives of this study were as follows:

1. Develop methodology to quantify the presence of nicotinamide hemochromes.
2. Prescreen various ligands in a ground turkey system for their ability to reduce pinking in control samples as well as pinking agent (nicotinamide and sodium nitrite) treated samples.
3. For those ligands that demonstrated a significant reduction in pinking, comprehensive testing was performed in whole turkey *Pectoralis major* muscles with and without nicotinamide and sodium nitrite as pinking agents. Ligand efficacy was determined at various ligand levels and over different periods of storage.

The three research chapters are presented as individual papers that will be submitted for publication.

References

Ahn, D.U. and Maurer, A.J. 1990. Poultry meat color: kinds of heme pigments and concentrations of the ligands. Poultry Sci. 69:157-165.

Akoyunoglou, J-H.A., Olcott, H.S., and Brown, W.D. 1963. Ferrihemochrome and ferrohemochrome formation with amino acids, amino acid esters, pyridine derivatives and related compounds. Biochem. 2:1033-1041.

Brown, W. D. and Tappel, A.L. 1957. Identification of the pink pigment of canned tuna. Food Research. 22:214-219.

Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., and Bartholomew, D.T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51:1132-1135.

Dobson, B.N. and Cornforth, D.P. 1992. Nonfat dry milk inhibits pink discoloration in turkey rolls. Poultry Sci. 71:1943-1946.

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 contnet of each chromoprotein to body mass. J. Biol. Chem. 182:317-333.

Froning, G.W. and Hartung, T.E. 1967. Effect of dietary nitrates and nitrites on color of chicken meat. Poultry Sci. 46:1261.

Froning, G.W., Daddario, J., Hartung, T.E., Sullivan, T.W. and Hill, R.M. 1969a. Color of poultry meat as influenced by dietary nitrates and nitrites. Poultry Sci. 48:668-674.

Froning, G.W., Mather, F.B., Daddario, J., and Hartung, T.E. 1969b. Effect of automobile exhaust fume inhalation by poultry immediately prior to slaughter on color of meat. Poultry Sci. 48:485-487.

Ghorpade, V.M. and Cornforth, D.P. 1993. Spectra of pigments responsible for pink color in pork roasts cooked to 65° or 82°C. J. Food Sci. 58:51-52, 89.

Girard, B., Vanderstoep, J., and Richards, J.F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55:1249-1254.

Hornsey, H.C. 1956. The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 7:534-540.

Hunt, M.C. and Hedrick, B. 1977. Chemical, physical, and sensory characteristics of bovine muscle from four quality groups. J. Food Sci. 42:716-720.

Livingston, D. J. and Brown, W. D. 1981. The chemistry of myoglobin and its reactions. Food Tech. 35:244-252.

Mugler, D.J., Mitchell, J.D., and Adams, A.W. 1970. Factors affecting turkey meat color. Poultry Sci. 48:1510-1513.

Niewiarowicz, A.., Pikul, J., and Czajka, P. 1986. Gehalt an myoglobin und hamoglobin in fleisch verschiedener geflugelarten. Fleischwirtschaft 66:1281-1282.

Pikul, J., Niewiarowicz, A., and Kupijaj, H. 1986. The cytochrome c content of various poultry meats. J. Sci. Food Agric. 37:1236-1240.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. 31:68,69,72-44.

Richardson, M., Posati, L.P., and Anderson, B.A. 1980. Composition of foods. Sausage and luncheon meats (Raw, Processed, Prepared). Handbook No. 8-7. United States Department of Agriculture, Science and Education Administration. Washington D.C.

Shaw, D.E., Claus, J.R., and Stewart, K.K. 1992. Effects of ammonia exposure on fresh pork: a distinct pink color after cooking. J. Muscle Foods 3:169-174.

Topel, D.G., Merkel, R.A., Mackintosh, D.L., and Hall, J.L. 1966. Variation of some physical and biochemical properties within and among selected porcine muscles. J. Anim. Sci. 25:277-282.

Chapter 2

Literature Review

2.1 Native Muscle Pigments

2.1.1 Hemoglobin

Hemoglobin is a tetrameric, globular heme protein (68,000 molecular weight) that functions to carry oxygen to the body's cells (Van De Graaff and Fox, 1989). Because hemoglobin is localized in the red blood cells, most of it is lost at slaughter during exsanguination. It is believed that if the exsanguination process is not adequate, the hemoglobin of residual blood may be available to color the muscle.

In an effort to minimize residual blood in the muscle, studies have been conducted to evaluate the effects of slaughter techniques on the completeness of blood loss. Ogielski and Wartenburg (cited in Warriss, 1977) found that the amount of residual blood in muscles increased as time between stunning and bleeding increased. The severity of blood splashing was enhanced as the time between stunning and exsanguination increased (Burson et al., 1983). Blood splashing is a defect that occurs when vessels in the muscle rupture and allow blood to penetrate the surrounding tissue. If blood splashing is severe, this defect can lead to significantly higher muscle hemoglobin concentrations. A study by Burson et al. (1983) found that captive bolt stunning in swine caused a higher incidence of blood splashing than with electricity or carbon dioxide. Warriss and Leach (1978) found that electrically stunned sheep lost more blood than those stunned by captive bolt and those bled lying lost more than those bled hanging. However, the residual hemoglobin

content of the muscle did not vary significantly between the slaughter practices examined. Warriss (1977) pointed out that the quantity of blood lost at slaughter did not include the blood remaining in the viscera, skin, and other inedible parts of the body. As a result, measurements of exsanguination blood loss could not be used as an accurate index of residual blood.

Since there are thousands of capillaries throughout muscle tissue, it would be unrealistic to believe that all blood could be removed from the muscle during exsanguination. Warriss (1977) reported that, generally upon the completion of exsanguination (in any species), about 60% of the blood was lost by bleeding, 20-25% remained in the viscera, 5-10% was found in the fat and bone, and not more than 10% was found in the musculature. Fox (1966) found that even in a well bled sample, hemoglobin may comprise 20-30% of the total heme pigments. Fleming et al. (1960) found hemoglobin to be 5% of the total pigment in beef rib eyes. The fact that hemoglobin exists in significant quantities within the muscle makes it a contributing factor to meat color that cannot be ignored. Table 1 lists reported hemoglobin, myoglobin, and cytochrome c levels in chicken, turkey, beef, and pork muscles. A general trend for each species was that myoglobin concentrations were the highest followed by hemoglobin and then cytochrome c. Poultry was the exception in that hemoglobin was similar or slightly higher in concentration than myoglobin.

Table 1 - Species and Muscle Differences in Myoglobin and Cytochrome c Level

Species/muscle	Pigment	Level (mg/g)	Reference
Chicken			
Light meat	myoglobin	0.06	Lawrie, 1950
		0.15	Fleming, 1990
	hemoglobin	0.17	Fleming, 1990
	cytochrome c	0.011	Pikul et al., 1986
		0.013	Fleming, 1990
Dark meat	myoglobin	0.60	Lawrie, 1950
		0.21	Fleming, 1990
	hemoglobin	0.38	Fleming, 1990
	cytochrome c	0.036	Pikul et al., 1986
		0.037	Fleming, 1990
Turkey			
Light meat	myoglobin	0.58	Niewiarowicz et al., 1986
		0.18	Fleming, 1990
	hemoglobin	0.63	Babji et al., 1982
		0.25	Fleming, 1990
	cytochrome c	0.013	Pikul et al., 1986
		0.013	Niewiarowicz et al., 1986
Dark meat	myoglobin	2.15	Pikul et al., 1986
	cytochrome c	0.047	Pikul et al., 1986
Beef			
Longisimus dorsi	myoglobin	3.42	Hunt and Hedrick, 1977
		3.97	Rickansrud and Henrickson, 1967
		4.79	Warriss and Rhodes, 1977
	hemoglobin	0.44	Warriss and Rhodes, 1977
	cytochrome c	0.015[a]	Drabkin, 1950
Psoas major	myoglobin	2.40	Rickansrud and Henrickson, 1967
		3.87	Warriss and Rhodes, 1977
	hemoglobin	0.65	Warriss and Rhodes, 1977
Pork			
Longissimus dorsi	myoglobin	2.87	Topel et al., 1966
		1.30	Lawrie, 1950
	hemoglobin	0.0008	Hazell, 1982
	cytochrome c	0.052	Girard et al., 1990
Psoas major	myoglobin	6.37	Topel et al., 1966
		2.80	Lawrie, 1950
	cytochrome c	0.081	Girard et al., 1990

[a] Cytochrome c level based on entire muscle mass of heifers not a specific muscle.

2.1.2 Myoglobin

Myoglobin is a monomeric globular heme protein with a molecular weight of about 18,000. Myoglobin is localized in the small red fibers of the muscle (Livingston and Brown, 1981) and it performs the physiological function of oxygen transfer from hemoglobin at the cellular level. Like hemoglobin, the concentration of myoglobin within the muscle cells is also important in determining muscle color. Myoglobin and hemoglobin are identical in their reactions, except that the reaction rates involving ligands, autoxidation, and denaturation are different for each pigment (Fox, 1966). Myoglobin concentration has been shown to vary widely with species, different muscles of the body (Rickansrud and Hendrickson,1967), muscle activity, age (Nishida, 1976) and sex of the animal (Froning et al., 1968). The differing levels of myoglobin between species and different muscles of the same animal are very pronounced (Table 1). The bovine Longissimus *thoracis et lumborum* muscle contains about 4.06 mg/g myoglobin while the psoas major contains about 3.14 mg/g myoglobin. Myoglobin concentration may even vary within the same muscle. The bovine inner S*emitendinosus* muscle contains 3.27 mg/g myoglobin while the outer S*emitendinosus* contains 2.42 mg/g myoglobin (Hunt and Hedrick, 1977). Pork has a myoglobin level of 6.37 mg/g in the P*soas major* and 2.87 mg/g in the Longissimus *thoracis et lumborum* (Topel, et al., 1966). Sheep have a muscle myoglobin content ranging from 4.5 to 5.5 mg/g (Ledward and Shorthose, 1971). Light meat tuna contains 0.5 to 1.0 mg/g myoglobin (Brown, 1962). The turkey P*ectoralis major* muscle contains about 0.58 mg/g myoglobin (Niewiarowicz et al., 1986) while dark

leg muscle contains 2.15 mg/g (Pikul et al., 1986). Lawrie (1950) reported that chicken thighs contain about ten times more myoglobin than the breast.

In considering age and sex of the animal, generally as an animal increases in age the myoglobin content also increases. Nishida (1976) reported the myoglobin content in the chicken leg, heart, and gizzard doubled between 6 and 27 weeks of age. Froning et al. (1968) reported a similar trend for turkeys. Froning et al. (1968) also found that both raw and cooked turkey meat had higher Gardiner 'a' values (more red) with an increase in age, indicating a higher myoglobin content. In addition, muscles from males (0.50 mg/g) turkeys had significantly higher myoglobin concentrations than females (0.20 mg/g; Froning et al., 1968). As with age, the Gardiner 'a' values for both raw and cooked turkey were higher for males than females. High levels of muscular activity may evoke the generation of more myoglobin in muscles (Lawrie, 1991) possibly because more active muscles have higher oxygen requirements. This was clearly illustrated by the higher myoglobin content of poultry leg and thigh as compared to the breast.

2.1.3 Cytochrome c

Cytochrome c, like myoglobin and hemoglobin, is a heme iron containing pigment and has a molecular weight of ca 13,000 (Lehninger et al., 1993). Cytochrome c is located in the inner mitochondrial membrane and has the primary function of electron transfer from the cytochrome reductase complex to cytochrome oxidase during cellular respiration (Lehninger et al., 1993). Levels of cytochrome c are more consistent across species and muscles than myoglobin (Table 1). In pork, the level of cytochrome c was

slightly lower in the *longissimus dorsi* (0.052 mg/g) than the *psoas major* (0.081 mg/g; Girard et al., 1990). The same trend followed in chicken where the levels of cytochrome c were 0.013 and 0.037 mg/g in chicken light and dark meat, respectively (Fleming, 1990). Cytochrome c was present in turkey light and dark meat at 0.013 and 0.047 mg/g, respectively (Pikul et al., 1986). Dark muscles in poultry and muscles like the *psoas major* in pork have higher aerobic capabilities and therefore a more developed oxidative system of mitochondria and cytochromes (Girard et al., 1990).

Unlike myoglobin, cytochrome c concentration was found to be inversely proportional to age because the increase in mitochondria number may lag behind the increase in sarcoplasmic volume that occurs with fiber growth (Girard et al., 1990). In addition, the levels of cytochrome c in muscle are considerably lower than the levels of myoglobin (Table 1). Akeson et al. (1960) reported the concentration of cytochrome c in rat muscle to be 1/50 to 1/100 that of myoglobin.

The extinction coefficient of myoglobin at wavelengths in the pink/red area of the spectrum is 11.8 for the sperm whale and 13.8 for the horse (Antonini and Brunori, 1971). The extinction coefficient for human hemoglobin is 12.5 at 555 nm (Antonini and Brunori, 1971). Ahn and Maurer (1989a) reported work by Van Gelder and Slater (1962) who found the extinction coefficient of cytochrome c at 550 nm to be three fold higher than the extinction coefficient of myoglobin. Because the extinction coefficient has the units of absorbance/concentration/length, the slope of cytochrome c on an absorbance verses concentration plot would be about three times as steep as the slope of myoglobin. In

poultry products where the ratio of cytochrome c to myoglobin is lower, cytochrome c may make a significant contribution to color.

2.2 Heme Pigment Structure and Chemical States

2.2.1 Heme pigment structure

Heme pigments all share a basic structure in that they are comprised of a protein and non-protein subunit. The protein moiety is called the globin and consists of ca 574 amino acids between the four chains of hemoglobin, 160 amino acids in myoglobin (Antonini and Brunori, 1971), and 103 amino acids in cytochrome c (Moore and Pettigrew, 1990). The number of amino acids for these pigments may vary slightly between species. Unlike myoglobin and hemoglobin, where 80% of the protein is globular, the protein of cytochrome c is much more compact with only 20% being globular (Margoliash, 1963). The globin amino acids of heme pigments align themselves in a way such that a hydrophobic pocket is created. It is this pocket that houses the second subunit, the heme group. The compact nature of the cytochrome c globin shields the heme group, which is housed in the center of the molecule, to a greater degree than the globin of myoglobin and hemoglobin (Margoliash and Bosshard, 1963).

The heme group (Figure 1) consists of a planar porphyrin ring which is made up of four pyrrole rings attached to each other through methine bridges (Clydesdale and Francis, 1971). The conjugated nature of the porphyrin ring allows the pi electrons of the double bonds to resonate around and stabilize the ring (Cornforth, 1989). Bound to the center of

porphyrin ring is a molecule of iron. It is the iron of the heme group that makes interactions with various ligands possible.

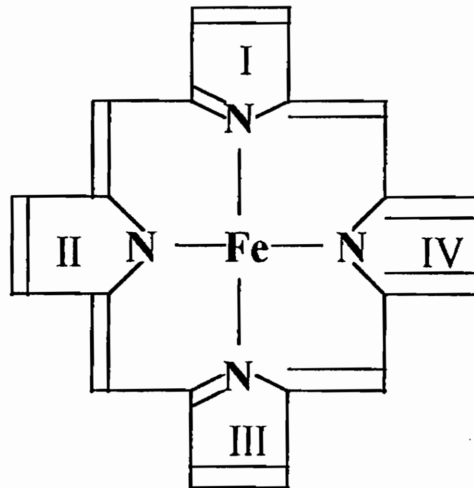


Figure 1- Structure of Heme Group (Kagen, 1973)

2.2.2 Iron oxidation states

The crucial component of the heme group, with respect to physiological functionality and color forming reactions, is the iron molecule. The oxidation state and the type of ligand bound to the iron determines the color and reactivity of the pigment under most reaction conditions (Livingston and Brown, 1981). The electron distribution of iron is very important to its activity. Iron loses two or three of its electrons because of its low electronegativity (Livingston and Brown, 1981) and thus can exist in the two common oxidation states (figure 2) referred to as ferrous or reduced iron (+2) and ferric or oxidized iron (+3). Ferrous iron consists of an Argon core plus six 3d electrons while

ferric iron has an Argon core and only five 3d electrons (Giddings, 1977a). The oxidation state of the iron in all three heme pigments is crucial in determining what ligand, if any, binds to the heme.

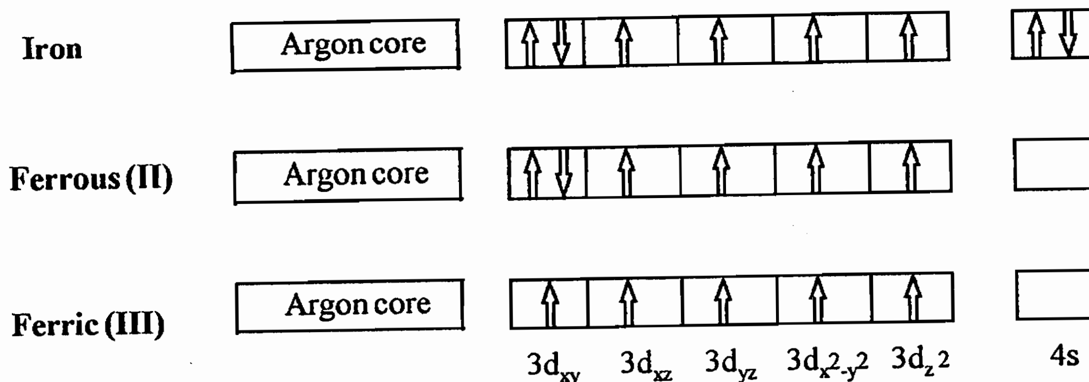


Figure 2- Electron Distribution of Iron (Giddings, 1977a)

2.2.3 Nature of the heme-ligand bond

The coordination chemistry of iron is such that it complexes with up to six donor ligands by electrostatic (ionic) or covalent interactions depending on the electron donating potential of the ligand (Giddings, 1977a). Four of the six binding sites are occupied by the pyrrole nitrogen atoms of the porphyrin ring (Antonini and Brunori, 1971) leaving only the fifth and sixth sites of iron to react with other ligands. A ligand that is less likely to share its electrons will form an ionic bond and an atom that is more likely to share its electrons will form a covalent bond (Price and Schweigert, 1971). Also, in the absence of strong covalent complexers, ionic compounds can be formed with molecules like water (Clydesdale and Francis, 1971). Water can bind to iron through its oxygen atom and since water is not as strong of an electron pair donor as molecular oxygen, an ionic complex

results (Clydesdale and Francis, 1971). Upon the availability of covalent complexers like oxygen, nitric oxide, carbon monoxide, and cyanide, the weaker ionic bond will be broken and a more stable covalent bond will form (Clydesdale and Francis, 1971). According to Price and Shweigert (1971), the type of bond formed is the most important factor in determining the final character and color of the complex. The next most important factors are the oxidation state of the iron and the physical state of the globin.

Within a covalent complex between heme iron and a ligand there are two types of bonds formed. An example of these bonds is shown for the oxymyoglobin complex in Figure 3. The sigma (σ) bond is formed by the donation of electrons from the ligand to

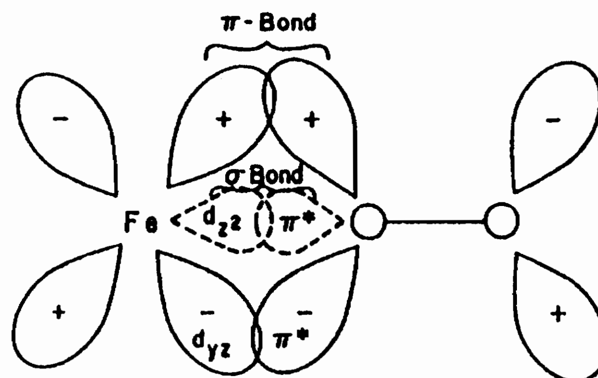


Figure 3- Bonding Geometry for the MbO_2 Complex (Livingston and Brown, 1981)

the iron cation (Livingston and Brown, 1981). Increasing electronegativity of the ligand enhances its reactivity with the heme group, provided that steric factors do not prevail, because the ligand is better able to donate electrons (Akoyunoglou et al., 1963). The other type of bonding in a covalent complex is pi (π) bonding; sometimes referred to as

back bonding. In π bonding the iron donates electrons back to the ligand via the ligand's π or π^* orbitals. This type of bonding is especially important in the oxymyoglobin complex because oxygen is a fairly weak σ donor. For π bonding to occur the iron must have sufficient electron density to donate to its ligand. Ferrous iron meets this criterion but ferric iron does not because of its high nuclear charge (Livingston and Brown, 1981). This is one reason why there are ligand binding differences between ferrous and ferric heme pigments.

Once a ligand is bound to iron, the electron donating/withdrawing power of the ligand is transmitted across the porphyrin ring via the conjugation and influences the basicity of the central pyrrole nitrogens (Giddings, 1977a). This in turn affects the electromagnetic properties of the iron and exerts what is known as the "cis effect" upon axial ligands. The stronger the porphyrin to iron bonding the weaker the iron to axial ligand bond at the fifth and sixth positions and vice versa (Giddings, 1977a). Likewise, greater porphyrin basicity (electron donor strength) imparts a greater π donor ability to iron for bonding axial ligands to the fifth and sixth positions (Giddings, 1977a). This results in an increased ability of iron to bind pi acceptor/sigma donor ligands like O_2 and NO (Giddings, 1977a). The crystal field theory states that for an iron cation surrounded by six ligands, the five d electron levels will be divided into a group of higher and a group of lower energy levels (Livingston and Brown, 1981). The way the iron d electrons fill up the energy levels depends upon the surrounding ligands (Livingston and Brown, 1981). A representation of energy levels is shown in Figure 4. The high spin derivatives have many

unpaired electrons and are termed paramagnetic; meaning they are attracted by a magnetic field. Low spin molecules with no unpaired electrons are a termed diamagnetic and are repelled by a magnetic field. Two paramagnetic molecules such as deoxymyoglobin and

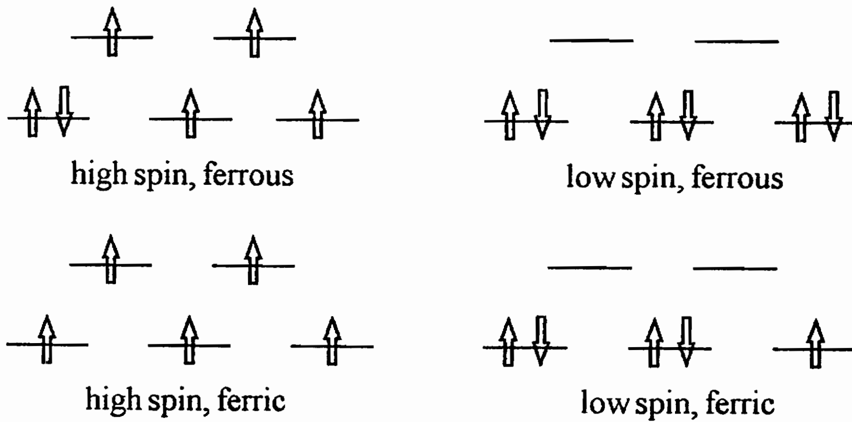


Figure 4- Energy Level Diagrams for 3d Electrons (Livingston and Brown, 1981)

molecular oxygen can combine to form a diamagnetic compound (e.g. oxymyoglobin). Once formed any diamagnetic substance will have less tendency to react with a paramagnetic species because there are no unpaired electrons to react (Livingston and Brown, 1981). Likewise, a paramagnetic complex may also be formed. Such a complex, like nitric oxide myoglobin, would be unstable and react with another paramagnetic species (Livingston and Brown, 1981).

2.3 Ligands that Bind Heme Pigments

2.3.1 Ferrous and ferric derivatives of native myoglobin and cytochrome c

Ferrous derivatives of native myoglobin

The two most common derivatives of ferrous myoglobin are deoxymyoglobin and oxymyoglobin. Deoxymyoglobin is represented by the Fe(II) energy levels in Figure 5. Deoxymyoglobin is the only ferrous derivative that does not have a sixth ligand attached to the iron. Having only five ligands alters the myoglobin's electronic structure from the crystal field theory by making it a high spin, paramagnetic species (Livingston and Brown, 1981). In the absence of atmospheric oxygen myoglobin is principally in the deoxy form and imparts a purple color to meats (Cornforth, 1989). This purple color results because deoxymyoglobin has an absorption band in the green portion of the spectrum (Clydesdale and Francis, 1971).

Since deoxymyoglobin is paramagnetic, it to reacts with another paramagnetic species, molecular oxygen, to form oxymyoglobin MbO_2,(Figure 5). Together, the two form a diamagnetic compound which is no longer reactive with paramagnetic species as long as they remain complexed (Livingston and Brown, 1981). When myoglobin is oxygenated it gives meat a reddish color. This is most apparent in the bright cherry red color of fresh beef. The overall electron distribution of this complex is at a lower energy state than either deoxymyoglobin or oxygen which makes the MbO_2 complex very stable (Livingston and Brown, 1981). In the presence of oxygen the three myoglobin forms (deoxymyoglobin, oxymyoglobin, and metmyoglobin) are constantly interconverting (Fox, 1966). This interconversion is referred to as the color cycle

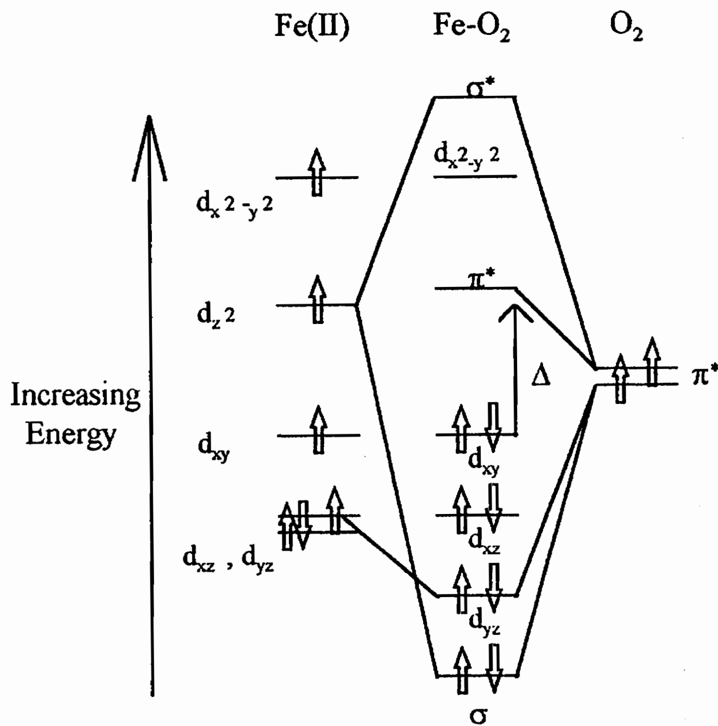


Figure 5- Energy Level Diagram for MbO_2 Complex (Livingston and Brown, 1981)

(Clydesdale, 1971). For oxygen to bind reversibly to myoglobin at the sixth site an effective fifth ligand must first be coordinated to iron so that a square pyramidal complex is formed (Giddings, 1977b). This shape favors the release of oxygen which is necessary to provide oxygen to the tissue. The imidizole of a histidine residue of the globin in myoglobin and hemoglobin is an especially effective fifth ligand because it is a good π donor (Giddings, 1977b). This helps the iron engage in back bonding of electrons to the oxygen. A less effective base than histidine would increase the possibility of oxidation rather than oxygenation of myoglobin while a stronger base would make oxygen binding less reversible (Giddings, 1977b).

Any atom having an electron pair to donate can bind to the heme iron (Price and Schweigert, 1971), if it is sterically favorable. Because of the globin protein's configuration around the heme of myoglobin, only small ligands can bind to the iron (unless the globin is opened by denaturation). Carbon monoxide (CO) has the ability to donate electrons and it is small enough to interact with the heme group. Like oxymyoglobin, carboxymyoglobin is red in color because the similar binding pattern causes absorption of light of the same wavelengths (Livingston and Brown, 1981). In addition, carboxymyoglobin is a diamagnetic compound so it is stable to attack from paramagnetic molecules while complexed. The binding of carbon monoxide is different from oxygen in that the bond is more sigma in character. This allows the association between the two to remain bonded through globin denaturation (Livingston and Brown, 1981). Carbon monoxide also dissociates from myoglobin about 1000 times more slowly than oxygen because the complex is so stable (Livingston and Brown, 1981).

Another ligand that binds to ferrous myoglobin well and has been utilized for years in the curing process is nitric oxide (NO). Nitrates and nitrites are converted to nitric oxide so that they can react with myoglobin. Nitric oxide myoglobin (NOMb) is a bright red derivative with a spectrum similar to oxymyoglobin. The bonding of NO to iron is via σ and π bonds (Livingston and Brown, 1981). NO dissociates from myoglobin approximately a million times slower than oxygen but the complex is still considered unstable in the presence of oxygen. When NO does dissociate, oxygen, which is present in much higher concentrations, will take its place on the myoglobin. In addition, in the presence of oxygen NO is converted to NO_2 (Giddings, 1977b). NOMb is the only

common ferrous derivative, besides deoxymyoglobin, that is paramagnetic. This means that it can react with other paramagnetic molecules where a diamagnetic species like oxymyoglobin would be inert (Livingston and Brown, 1981). This contributes to the instability of NOMb and the only way to stabilize it is to denature the complex (curing process).

Ferrous derivatives of native cytochrome c

Native ferrocytochrome c, unlike myoglobin, does not react with oxygen or many other ligands due to shielding of the heme by the globin. The great affinity of the bivalent iron for nitrogenous groups provided by amino acid residues in the globin and the nature of the protein to fold itself also decrease native ferrocytochrome c's reactivity toward external ligands (Keilin, 1966). As with myoglobin ligands, strong cytochrome c ligands are good σ donors and π acceptors. CN^- and NO are particularly efficient in this area. Weak donor anions like halides and hydroxyl radicals and neutral saturated molecules like NH_3 and H_2O are poor ferrocytochrome c ligands (Moore and Pettigrew, 1990). Carbon monoxide does not react with native ferrocytochrome c (Keilin, 1966). NO reacts with ferrocytochrome c (Keilin, 1966) to form a compound that produces a red color similar to NO myoglobin.

Ferric derivatives of native myoglobin

Deoxymyoglobin and oxymyoglobin can undergo a process known as autoxidation in which the ferrous iron loses another electron to produce metmyoglobin (fe+3). Autoxidation occurs at low partial pressures of oxygen which causes some of the MbO_2 to deoxygenate. The stability of the oxymyoglobin/deoxymyoglobin mixture against

oxidation is greatly diminished as compared to all of the myoglobin being bound to oxygen (Livingston and Brown, 1981). Oxygen, a high field ligand, protects myoglobin from oxidation because the diamagnetic complex is so stable (Livingston and Brown, 1981). If oxymyoglobin undergoes oxidation to metmyoglobin the oxygen dissociates and cannot bind again until the iron is reduced to the ferrous state complex (Livingston and Brown, 1981). Water becomes bound to metmyoglobin by sigma bonding to form a reactive paramagnetic complex (Livingston and Brown, 1981). The water bond is ionic in nature and can be replaced by covalent bonding when a high field ligand is available. Oxidation of ferrous iron occurs at low oxygen pressures with the rate being fastest at 1-1.4 parts per million oxygen pressure (George and Strattman, 1952). Metmyoglobin has an absorption peak towards the blue end of the spectrum (505 nm) which gives the compound a resultant brown color.

The high nuclear charge of ferric iron does not engage in strong back bonding so oxygen can't bind as it would with ferrous iron (Livingston and Brown, 1981). In general an electron donor will combine with ferrous iron if it is neutral (O_2 , NO, CO) and with ferric iron if it is negatively charged (CN^-, OH^-, N_3^-) (Price and Schweigert, 1971). Ligands such as CN^-, NO, and N_3^- are superior electron donor atoms so they can compensate for the lack of ferric iron π donorship (Giddings, 1977b). It has also been speculated that a negatively charged ligand will neutralize the 'extra' positive charge that ferric iron carries (Price and Schweigert, 1971).

Of the ligands discussed, only nitric oxide can bind to the ferric iron, even though it is neutral, as well as ferrous iron. NO can bind ferric iron because of its superior σ

donating character (Livingston and Brown, 1981). The resulting complex is paramagnetic, like the ferrous-NO complex, but to a greater degree. This makes nitrosylmetmyoglobin very reactive and unstable, especially in air (Livingston and Brown, 1981).

Ferric derivatives of native cytochrome c

Ferrocytochrome c, unlike myoglobin, is not autoxidizable (Keilin, 1966). Thus, it does not lose of an electron at low oxygen partial pressures to form ferricytochrome c because this compound has no reactivity with oxygen. Under oxidizing conditions or with the assistance of oxidizing agents ferricytochrome c is formed. Ferricytochrome c is not as stable as its ferrous counterpart (Keilin, 1966) which is attributable to a destabilizing effect of the increased positive charge. Ferricytochrome c can react with CN^- at physiological pH (Keilin, 1966) because the negative charge can partially neutralize ferric's positive and because CN^- is an excellent electron donor. As with myoglobin, NO can bind the ferric cytochrome c derivative as well as the ferrous. Carbon monoxide, however, can not react with cytochrome c.

2.3.2 Ferrous and ferric derivatives of denatured myoglobin and cytochrome c

Ferrous derivatives of denatured myoglobin

Upon denaturation, the possible ligands that can combine with ferrous myoglobin are limited only by their availability. Once the globin unfolds and exposes the fifth and sixth binding sites on iron, a multitude of interactions can take place. Oxygen does not bind well to denatured ferrous myoglobin because there are many other ligands available

that are better electron donors. Carbon monoxide ferromyoglobin is a very stable compound. Because of this stability, carbon monoxide can remain bound to the heme through denaturation to yield carbon monoxide ferrohemochrome (Livingston and Brown, 1981). Even if carbon monoxide did become dissociated it would most likely rebind to the denatured pigment (Livingston and Brown, 1981). The diamagnetic nature of the CO-Mb complex adds to its stability. In contrast, the paramagnetism of the NO-Mb complex makes it an unstable compound and denaturation is necessary to stabilize it. When the globin of NO-Mb is denatured, the histidine residue attached to the fifth site is labilized and replaced by a second NO (Livingston and Brown, 1981). The resulting di-NO-heme compound is a diamagnetic complex which is very stable and unreactive (Livingston and Brown, 1981).

Denaturation of the globin has more effects than simply exposing the heme group. As the globin is being denatured so are other meat proteins. Many of these denatured proteins have free alpha amino groups while others are broken down to free amino acids. These alpha amino groups (if their hydrogens are not substituted) and free amino acids can coordinate with iron to form hemochromes (Livingston and Brown, 1981). The imidizole group of histidine is a particularly good ligand (Ahn and Maurer, 1990a) and other amino acids like lysine and methionine have potential as well.

Another potential ligand of denatured ferromyoglobin is nicotinamide. Nicotinamide is present in significant amounts in turkey (0.083 mg/g niacin; Richardson et al., 1980) and to a lesser degree in pork (0.049 mg/g niacin; Anderson, 1992) and beef (0.039 mg/g niacin; Anderson and Hoke, 1990). The nitrogen of this substituted pyridine

ring binds particularly well to the denatured ferrous iron. If available, other nitrogenous compounds like pyridine, substituted pyridines, and pyrazine, will bind to iron. Ahn and Maurer (1990a) found that nicotinamide and pyridine bind strongly to produce a large absorbance spike in the 560-570 nm range.

Ferrous derivatives of denatured cytochrome c

Cytochrome c undergoes a character change when it is denatured. Interaction with many ligands that could not bind native cytochrome c can occur after denaturation. The heme group is no longer shielded and many of the globin interactions that made it so stable are broken. Denatured ferrocytochrome c becomes able to bind with carbon monoxide. In addition, denatured ferrocytochrome c is autoxidizable, meaning it can be oxidized by oxygen to the ferric state (Keilin, 1966). Ahn and Maurer (1990a,c) found that denatured ferrocytochrome c could form ferrohemochromes with pyridine, histidine, nicotinamide, methionine, and cysteine. It was also found that heme complex forming reactions are much stronger for cytochrome c and the resulting complexes are much more stable than myoglobin complexes (Ahn and Maurer, 1990a).

Ferric derivatives of denatured myoglobin

Ferrihemochromes of myoglobin can be formed by the denaturation of metmyoglobin in which the water is replaced by another sixth ligand that is a better σ donor (Livingston and Brown, 1981). Alternatively, ferrohemochromes can easily be oxidized to ferrihemochromes (Livingston and Brown, 1981) and a constant interconversion between the two takes place. Many of the same ligands that bind ferrous

iron also bind ferric iron. Among these are NO , pyridine, and nicotinamide (Livingston and Brown 1981).

Ferric derivatives of denatured cytochrome c

The derivatives of denatured ferricytochrome c are quite similar to those of denatured ferric myoglobin. A strong electron donor is required because of the poor donating capacity of ferric iron. The conditions of the environment influence whether the pigment will be in the ferrous or ferric state and when it will interconvert. Strong field ligands like nicotinamide and pyridine can form ferricytochrome c hemochromes (Ahn and Maurer, 1990a).

2.3.3 Spectra of common native and denatured heme complexes

When specific ligands bind to the different oxidation states of iron of the various heme pigments an absorbance spectrum unique to that pigment is created. The absorbance spectrum specifies the extent to which each wavelength of light is absorbed and therefore which colors will be visually perceived. Table 2 lists the wavelengths that correspond to reported absorbance peaks of the common heme pigment derivatives. The spectrum of visible light is from 400 to 700 nm in wavelength. Lehninger et al., (1993) cited that the visually perceived colors are violet (400 nm), blue (450 nm), green (500 nm), yellow (550 nm), orange (600 nm), and red (650nm). For the compounds in Table 1, the wavelengths absorbed are the ones that can not be visually perceived. Therefore many of the derivatives listed reflect a pink to red color because portions of the blue, green, and yellow spectra are absorbed.

Table 2- Absorbance Peaks Indicative of Common Heme Pigment Derivatives

Derivative	Absorbance (nm)	Reference
deoxymyoglobin	434	Ngoka and Froning, 1982
	555	Janky and Froning, 1973
	434, 556	Antonini and Brunori, 1971
oxymyoglobin	417, 543, 575	Girard et al., 1990
	545, 582	Janky and Froning, 1973
	418, 543, 581	Antonini and Brunori, 1971
metmyoglobin	406, 500, 630	Girard et al., 1990
	505, 627	Janky and Froning, 1973
	410, 505, 635	Antonini and Brunori, 1971
carbon monoxide myoglobin	423, 542, 579	Antonini and Brunori, 1971
	533, 563	Keilin, 1960
nitric oxide myoglobin	543, 575	Antonini and Brunori, 1971
nitric oxide metmyoglobin	530, 572	Antonini and Brunori, 1971
cyanide metmyoglobin	423, 540	Antonini and Brunori, 1971
fluorine metmyoglobin	406, 490, 609	Antonini and Brunori, 1971
N_3^- metmyoglobin	420, 540, 570	Antonini and Brunori, 1971
hemoglobin	430, 555	Antonini and Brunori, 1971
	439, 555	Girard et al., 1990
oxyhemoglobin	415, 541, 577	Antonini and Brunori, 1971
	417, 542, 577	Girard et al., 1990
methemoglobin	405, 500, 631	Antonini and Brunori, 1971
carboxyhemoglobin	419, 540, 569	Antonini and Brunori, 1971
nitric oxide hemoglobin	545, 575	Antonini and Brunori, 1971
cyanide methemoglobin	419, 540	Antonini and Brunori, 1971
fluorine methemoglobin	403, 483, 605	Antonini and Brunori, 1971

Table 2- continued

Derivative	Absorbance (nm)	Reference
N_3^- methemoglobin	417, 540, 575	Antonini and Brunori, 1971
imidizole methemoglobin	411, 534, 560	Antonini and Brunori, 1971
ammonia methemoglobin	411, 535	Antonini and Brunori, 1971
ferrocytochrome c	415, 485	Keilin, 1966
	414, 520, 550	Girard et al., 1990
	520, 550	Ahn thesis, 1988
ferricytochrome c	409, 498	Keilin, 1966
cyanide ferricytochrome c	413, 501	Keilin, 1966
N_3^- ferricytochrome c	411, 501	Keilin, 1966
NO ferricytochrome c	417, 475	Keilin, 1966
histidine Mb hemochrome	530, 560	Ahn and Maurer, 1990a
pyridine Mb hemochrome	530, 564	Ahn and Maurer, 1990a
nicotinamide Mb hemochrome	528, 558	Ahn and Maurer, 1990a
	527, 555	Howard et al., 1973
nitrosylhemochrome	540, 565	Cornforth et al., 1986
denatured globin hemochrome	558	Cornforth et al., 1986
	424, 527, 557	Tappel, 1957
histidine cyt c hemochrome	522, 550	Ahn and Maurer, 1990a
pyridine cyt c hemochrome	520, 550	Ahn and Maurer, 1990a
nicotinamide cyt c hemochrome	520, 550	Ahn and Maurer, 1990a

2.4 Factors Effecting Ligand Binding and Ligand Availability

2.4.1 Pigment denaturation

Denaturation is the process by which the native protein portion (globin) of the heme pigments undergoes a structural degradation as a result of changes in the environment. In relation to hemoglobin, myoglobin and cytochrome c, denaturation is the conversion of the ferrous and ferric pigments to ferrohemochromes and ferrihemochromes, respectively. This conversion involves an unfolding of the native globin protein thereby exposing the heme (Livingston and Brown, 1981). As the definition implies, it is necessary to denature the protein to form a hemochrome (Fox et al., 1974).

Effect of temperature

It has long been understood that increasing the temperature can denature proteins. Some proteins, such as enzymes, are more sensitive to heat while others are more resistant but no protein is immune to the effects of heat. Many studies have been performed to define the temperatures of denaturation for hemoglobin, myoglobin, and cytochrome c. Some experiments measured pigment denaturation in a model solution system while others studied denaturation in the meat itself.

General guidelines for beef cookery dictate that if cooked to an internal temperature of less than 60°C, the product will be rare, 60°-70°C will be medium, and 70°-80°C will be a well done product (Bernofsky et al., 1959). However, achieving a product with a color that reflects the internal temperature is not as simple as monitoring that temperature because heme pigments denature at different temperatures and the

pigment environment has a notable effect on denaturation temperature. Bernofsky et al. (1959) found that there was no appreciable denaturation of myoglobin in a pure solution below 65°C and that myoglobin denaturation was considerable in ground beef at 65°C. The temperature at which one half of the heme pigments were denatured (1/2D) in solution was shown by Cornish and Froning (1974). Hemoglobin was 1/2D at 62°C, myoglobin at 78.5°C, and cytochrome c was not denatured even at 105°C in solution. Ahn and Maurer (1989b) reported that 90% of the hemoglobin in solution was denatured at 74°C while only 65% of the myoglobin and 75% of the cytochrome c were denatured at 85°C. Up until 80°C, the rate of myoglobin denaturation in solution was faster than the rate of cytochrome c denaturation but after 80°C more cytochrome c than myoglobin was denatured (Ahn and Maurer, 1989b). The heat resistance of cytochrome c was much lower here than reported by Cornish and Froning (1974). This could be because a lower buffer concentration was used here which may play an important role in decreasing cytochrome c stability (Ahn and Maurer, 1989b). Helmke and Froning (1971) found that as end-point temperature in cooked turkey increased above 60°C, the color lightened significantly as indicated by higher L values. In addition, Gardiner a values (redness) decreased as the end-point temperature increased beyond 71°C which indicated greater myoglobin denaturation (Helmke and Froning, 1971). In pork roasts myoglobin appeared to be completely denatured at internal temperatures between 70°-80°C (Howe et al., 1982).

Oxidation state of the heme pigments appears to affect the temperature of denaturation as well. Janky and Froning (1973) noted that metmyoglobin was more heat

stable than either deoxymyoglobin or oxymyoglobin in a model system. They hypothesized that the stability may be due to the increase in polarity of metmyoglobin caused by the positive charge on the heme iron. This was evidenced by 75.9% deoxymyoglobin denaturation, 60.8% oxymyoglobin denaturation, and 54.3% metmyoglobin denaturation at 90°C. They hypothesized that this was due to the increased polarity of metmyoglobin caused by the positive charge on the iron which in turn increased the solubility of the pigment in the water of the model. Draudt (1969) reported that metmyoglobin was the least stable form of myoglobin in a ground beef system but in purified form metmyoglobin was one of the most stable forms of myoglobin. The effect of oxidation state on cytrochrome c in solution was different than myoglobin in that ferrouscytochrome c was more heat stable than ferricytochrome c. One reason was that the iron in ferrocytochrome c was more tightly bound to the protein than the iron in ferricyochrome c therefore making it harder to unfold the protein from around the iron (Keilin, 1966).

When heated, ferrocytochrome c undergoes a reversible denaturation and loosening of its tertiary structure. In this state it becomes autoxidizable and can combine with carbon monoxide. Upon cooling, however, about 80% of the denatured ferrocytochrome c can revert to its original state (Keilin, 1966). Ferricytochrome c did not exhibit this regeneration probably because it is not as stable a structure. The regenerated (undenatured) cytochrome c could be a major contributor to color of meat products because undenatured heme pigments generally exhibit stronger colors (pink/red) than denatured heme pigments.

Generally, heme pigments are more heat stable in solution than in the meat itself. This suggests that there are pigment-meat constituent interactions that reduce the thermal stability of the heme pigments. Draudt (1969) believed that the pigment stability was lower in meat because of heme-protein complex formation with many of the proteins available in the meat system. Whatever the mechanism responsible, the interaction of the pigments with meat components has a heat stability lowering effect.

Effect of salt, phosphate and pH

Ahn and Maurer (1989b) found that adding salt to a myoglobin solution significantly decreased its heat stability at temperatures from 60°-85°C. The decrease in heat stability could be caused by the chloride ion. The exposure of the heme group by heating may have given the chloride ions a chance to react with the iron. Subsequent bond cleavage could have occurred to yield apomyoglobin and chlorohemin (Ahn and Maurer, 1989b). Salt reduced the heat stability of myoglobin in meat as well as solution as was evidenced by increased myoglobin denaturation when salt was added to ground beef (Trout, 1989). Unlike myoglobin, added salt increased the heat stability of cytochrome c when the solution was heated to 80°C or higher (Ahn and Maurer, 1989b).

In general, the heat stability of myoglobin derivatives decrease as the pH drops. Janky and Froning (1973) showed that percentage denaturation values at pH 5.5 were significantly higher than at pH 6.6. At pH 6.0 and greater, protein is protected from heat denaturation (Cornforth, 1989). Brown et al. (1962) attributed the lower heat stability at lower pH values to globin unfolding and partial loss of the alpha helix formation. In a sense, lower pH has a denaturation ability of its own. Trout (1989) reported that a high

pH (up to 7.0) had a similar protective effect on the myoglobin of beef, pork, and turkey. Added phosphate to a myoglobin solution increased the heat stability of myoglobin at 74°C or higher (Ahn and Maurer, 1989b). This effect was probably due to the raise in pH (0.4 units) that phosphate caused. pH, like oxidation state, had the opposite effect on cytochrome c denaturation. Heat stability of cytochrome c was actually reduced by the increase in pH related to the addition of phosphate (Ahn and Maurer, 1989b). In fact, the degree of irreversible heat denaturation of cytochrome c was increased as the pH went from 7.2 to 10.2 (Butt and Keilin, 1962 as cited by Ahn and Maurer, 1989b).

Effect of moisture

Hagerdal and Martens (1976) showed that removing water from the myoglobin at moisture contents below 30% lead to decreasing amounts of protein being irreversibly denatured. This resistance to heat denaturation may result from the formations of inter and intramolecular bonds where the water had previously resided, thereby making the structure more stable (Hagerdal and Martens, 1976). In addition, water itself brings about thermal transitions because it changes physical state with increasing heat. It then follows that a lack of water decreases those transitions. With 30-80% water content, myoglobin underwent complete and irreversible heat denaturation (Hagerdal and Martens, 1976). At water contents greater than 80%, myoglobin underwent complete and irreversible denaturation but there was an increase in transition temperature and transition heat. This indicates that the protein was stabilized by excessive water and required more heat to denature it (Hagerdal and Martens, 1976).

Sterics

No matter what conditions lead to the denaturation of myoglobin, the unfolding of the protein has the effect of exposing the heme to its surroundings which make oxidation and discoloration easier (Livingston and Brown, 1981). In the case of myoglobin, the heme group is located in a cleft of the globin and contacted by 25 amino acid residues of the protein. The position of the heme makes it relatively inaccessible to many ligands. The imidizole of histidine 64, valine 68 and phenylalanine 43 present the most steric hindrance for ligands (Akoyunoglou et al., 1963). A similar steric hindrance exists for cytochrome c but to a greater degree. When denatured, the steric factors that once inhibited many bulky ligands from binding the heme iron are removed. Steric factors of the ligand itself, such as 2 and 5 substituted pyridines, are not affected by the denaturation of the pigments. Ligands that have their binding sites sterically hindered cannot bind to the heme ring. In addition, upon denaturation the fifth and sixth ligands of iron become available to bind external ligands whereas only the sixth will be available in the native pigments.

2.4.2 Effect of pH, phosphates, and salt

Environmental pH affects the denaturation of heme pigments by stabilizing them at alkaline pH and making them more heat sensitive at acidic pH. In addition, the oxidation state of the heme iron and thus the reaction of the heme with ligands, is largely decided by the pH of the meat or solution environment (Ahn and Maurer, 1990b). At a high pH, the iron is predominantly in the ferrous state while lower pH promotes a ferrous to ferric conversion (Ahn and Maurer, 1990b). Stated differently, as the pH decreases, the rate of

conversion of metmyoglobin to reduced myoglobin decreases (Cutaia and Ordal, 1964). Also, by increasing the pH of a denatured, the electronegativity of amino acid side chains (from the globin or other proteins) are increased because of increased hydrogen ion dissociation (Ahn and Maurer, 1990b). Increased electronegativity makes the amino acids more reactive ligands of iron. Ahn and Maurer (1990b) reported that a pH above 6.4 was favorable for heme complex formation with all of the ligands that were added except pyridine which could complex when the pH was greater than 6.1. At pH levels greater than 7.4, heme complexes formed with available amino acids when no external ligands were added. In the same study, Ahn and Maurer (1990b) found that cytochrome c was not affected by pH in this manner with regard to heme complex formation. A high pH was not always favorable for cytochrome c complex formation and an optimum pH for cytochrome c was difficult to predict.

Processing conditions in the formation of meat products can lead to changes in the pH and affect the ligand binding to the heme pigments. If phosphate is added, as is sometimes the case in poultry carcass wash water, the pH is raised. Ahn and Maurer (1990b) found that addition of 0.1M phosphate to a pigment solution increased the pH by 0.4 units and in turn increased heme complex forming reactions. The pH of meat is not something that is fixed for every carcass of every species. The ultimate pH is 5.3-5.6 for beef and 5.6-5.8 for pork (Kinsman et al., 1994). The pH for poultry is somewhat higher in the 6.0-6.2 range. If the animal is stressed before slaughter, the post mortem pH drop can be lessened because there will be less glycogen stores in the muscle to be metabolized anaerobically and less lactic acid will be produced. This mechanism is known to be the

cause of dark cutting beef. However, Ngoka et al. (1982) found that turkeys allowed to struggle freely during slaughter had significantly lower pH values as well as lower glycogen levels than an anesthetized group. Ngoka and Froning (1982) found that turkeys exposed to preslaughter excitement were darker in color. Babji et al. (1982) also found that higher pH meat was darker and redder while lower pH meat was lighter and less red. The dark color may be accounted for by greater heme complexing due to a higher pH.

An additive to many further processed meat products that may effect ligand binding is salt. Not only does salt decrease the stability of myoglobin to heat but it also can help ligands become available for binding. Ahn and Maurer (1989b) found that 2.5% added salt solublized most of the myofibrillar proteins and sacoplasmic proteins. These proteins (amino groups and free amino acids) were then available to form complexes with the heme.

2.4.3 Oxidation-Reduction potential (ORP)

The oxidation-reduction potential (ORP) of a model solution or a meat product is critical in determining what ligands will bind to the heme group and how strong the interaction will be. The ORP of living muscle is about +250 mv and the ORP of meat is about -50 mv (Solberg, 1970). Cornforth et al. (1986) reported that at an ORP of -550mv or less, all of the heme iron was in the ferrous state. Ahn and Maurer (1989b) found that during refrigerated storage of cooked turkey, salt and/or phosphate could lower the redox potential. As would be expected the addition of oxidizing or reducing agents to meat will change the potential of the system to favor either ferrihemochrome or ferrohemochrome

formation, respectively. The ORP will determine the oxidation state of iron which will in turn influence the ligand that binds and, ultimately, the color perceived.

2.5 Chemical and Electronic basis for Visually Perceived Colors

2.5.1 Electronic nature of the heme and globin

The resonant electrons of the porphyrin ring have a wavelength termed the "DeBroglie wavelength". These electrons can absorb visible light photons of a similar wavelength which is the basis for the color of heme pigments (Cornforth, 1989). When the heme iron is in the ferrous state, a pink, red, or purple color can be formed. When in the ferric state, the stronger positive charge of the iron pulls the resonant electrons inward and shortens their DeBroglie wavelength. As a result, shorter wavelengths of light are absorbed and the color visualized is brown (Cornforth, 1989). It has been postulated that a breaking of the porphyrin ring may result in yellow or colorless myoglobin derivatives (Janky and Froning, 1973) because the electrons would no longer be able to resonate around the porphyrin ring.

2.5.2 Effect of bound ligands on color

When a ligand is bound to a heme pigment by covalent bonding the resulting color is pink to red (Clydesdale and Francis, 1971). Examples of this are oxymyoglobin, carboxymyoglobin, nitric oxide myoglobin, cyanmetmyoglobin, and denatured globin hemochromes. Water bound to the sixth site of metmyoglobin is attached by ionic bonding and this contributes to the formation of the brown color. The reason that ligand

binding affects the color of the heme complex in this manner is two fold. First, the electrons donated by the ligand can resonate around the porphyrin ring to affect the wavelength of light absorbed (porphyrin π electrons to iron transitions). Secondly, when the complex is formed, iron and the ligand share electrons as shown in Figure 5 for the oxymyoglobin complex. The delta (Δ) symbol represents a possible electronic transition which would occur by absorption of visible light. The exact wavelengths absorbed by this transition and thus the visible wavelengths that are transmitted contribute to the color the complex takes on (Livingston and Brown, 1981).

2.6 Pinking Defect

2.6.1 Location and type of products affected

The pinking defect is a sporadic problem that affects cooked, uncured meat products. The defect has been identified in turkey rolls (Cornforth et al., 1986, Claus et al., 1994), roasted turkey (Pool, 1956), pork roasts (Ghorpade and Cornforth, 1993 and Howe et al., 1982), broiler chicken (Nash et al., 1985), bratwurst (Ghorpade et al., 1992), and tuna (Brown and Tappel, 1957). The pink defect is by no means limited to these products but seems to occur more commonly in products manufactured from low myoglobin meats.

Pinking is generally observed on the surface of fresh cut meats like turkey rolls (Cornforth et al., 1986). In many cases the pink color has been found to be unstable. Shortly after a product is cut the pink color may fade as a result of exposure to air and light (Howe et al., 1982, Cornforth et al., 1986). This fading may be caused by the same

autoxidation and photo-oxidation that causes cured products to fade (Walsh and Rose, 1957). In autoxidation of cured meats, molecular oxygen extracts a proton from the nitrosylhemochrome (MbNO) leaving metmyoglobin and a free nitrite ion (NO_2^-) as products. Walsh and Rose (1957) proposed that the mechanism of photo-oxidation involved the formation of an activated MbNO* molecule when light was absorbed. Once activated, MbNO then can give up an electron to oxygen to become metmyoglobin and NO_2^-. Furthermore, a freshly cooked product that exhibits no pink may develop the defect with refrigerated storage (Claus et al., 1994). This is thought to be associated with the development of reducing conditions in the meat as storage time increases.

2.6.2 Principle pigments involved in pinking

Brown and Tappel (1957) identified the pink pigment in tuna as a mixed denatured globin nicotinamide hemochrome. In this study, reflectance spectra of commercial pink and laboratory formulated (with nicotinamide) tuna preparations were similar. Cornforth et al. (1986) identified reduced globin or nicotinamide hemochromes as being the cause of pink color in turkey rolls. These pigments were identified by reflectance spectroscopy and were observed to quickly oxidize back to a tan color with exposure to air and light.

Several researchers believe that nitrosylhemochrome, the cured color pigment, is responsible for the pinking defect. Nitrite and/or nitrate are believed to contaminate the meat by a variety of routes such as the diet and drinking water (Froning et al., 1969b), carcass chill water (Mugler et al., 1970), and from manufacturing equipment which had been previously used for cured products. Nitric oxide (NO) and carbon moxoxide (CO)

have also been suggested to generate a pink color. Sources of NO and CO include automobile exhaust fumes (Froning et al., 1969a) and oven gases (Pool, 1956).

It seems likely that several different pigments may be responsible for the pink discoloration at any given time. Even if undenatured pigments or nitrosylhemochromes are not identified in a given pink product, that does not mean that they can't be the cause in another product. When looking for the cause of pinking every pigment that could cause a pink color needs to be investigated.

2.6.3 Effect of end-point cooking temperature

The color of cooked meats may be related to, at least in part, the undenatured native pigments myoglobin, hemoglobin, and cytochrome c (Trout, 1989). Undenatured pigments can cause strong red colors when bound to the certain ligands (O_2, CO, NO). End-point temperature has a bearing on the pinkness of cooked meats in relation to undenatured pigments. Ghorpade and Cornforth (1993) examined the spectra of pigments in pork roasts cooked to 65°C and 82°C. Roasts cooked to 65°C exhibited a pink color which was determined by reflectance spectra to be caused by undenatured myoglobin. The spectra of roasts cooked to 82°C did not have the peaks characteristic of undenatured myoglobin which indicated that pink color was due to other pigments. Girard et al. (1990) reported that a pink color in turkey breast and pork loin cooked anywhere from 65° to 95°C was caused by cytochrome c. The presence of cytochrome c in the ferrous state was confirmed by transmission spectroscopy (on cooked turkey or pork samples) and polyacrylamide gel electrophoresis (sample was extracted cytochrome c

from cooked turkey or pork). It is possible that the high thermal stability and regeneration capabilities of cytochrome c make this native pigment a major contributor to pink discoloration.

In general, end-point temperature studies show that native muscle pigments are denatured as the temperature increases. This is apparent in a study by Helmke and Froning (1971) in which advancing end-point temperature lightened the color of cooked turkey. In addition, the Gardiner a (redness) value was noted to decrease as end-point temperature increased. In contrast, Claus et al. (1994) reported that increasing cooking temperatures for turkey rolls between 71° and 80°C resulted in increased CIE a* values as well as an increased panelist perception of pink. These results, however, may be due to the addition of 2% nicotinamide to the rolls and the subsequent formation of nicotinamide hemochromes.

2.6.4 Effect of nitrogenous contamination

The formation of nitrosylhemochrome, the cured color pigment, can be a major cause of pink discoloration. Ahn and Maurer (1987) found the amount of nitrite in raw turkey breast to be between 3.8 and 21 ppm. This is far more than the 1 ppm of nitrite that Ahn and Maurer (1985) reported to be enough to cause a pink color in turkey breast meat. Sodium nitrite added to turkey breast significantly increased the ‘a’ value, nitrosoheme pigments, and residual nitrite while it decreased the ‘b’ and ‘L’ values. The only difference found between 20 and 50 ppm sodium nitrite treatments was that the residual nitrite was higher in the 50 ppm treatment (Ahn and Maurer, 1989a). At 10 ppm

added nitrite the maximum nitrosoheme pigment appeared to be formed and 2 ppm nitrite was still found as residual nitrite (Ahn and Maurer, 1989a).

Froning and Hartung (1967) found the uncooked white meat from broiler chickens fed sodium nitrate (150, 300, 600 ppm for 9 weeks) and sodium nitrite (25 and 50 ppm for 9 weeks) to have higher Gardiner a values and higher visual color scores than a control group fed a normal diet. This may indicate that dietary nitrates and nitrites are somehow stored in the muscle and have the ability to form heme complexes with native pigments. Similarly, nitrite or nitrate from the drinking water of an animal may increase the incidence of nitrosoheme complexes (Froning et al., 1969b) although Mugler et al. (1970) found that added nitrate levels up to 450 ppm of the drinking water had no significant effects on turkey meat color. Mugler et al. (1970) also found that a minimum of 200 ppm nitrate in poultry carcass chill water tanks was enough to make turkey meat significantly redder. A noticeable and measurable pink color was formed in cooked chicken that was soaked in a 4°C water bath with as little as 3 ppm nitrite for 4.5 days (Nash et al., 1985). Nash et al. (1985) reported that as little as 6 ppm nitrite in chill ice was enough to cause a pink color in chicken leg and thigh after 6.5 days of storage.

For nitrate or nitrite to form a heme complex they must first be converted to nitric oxide in the meat (Froning et al., 1969b). Therefore, gaseous nitric oxide would be able to react with heme pigments in this manner to produce nitrosylhemochrome. Froning et al. (1969a) studied the effects of automobile fume inhalation by poultry prior to slaughter in relation to pink color in raw and cooked chicken. Exhaust fumes contain nitric oxide, nitrogen dioxide, and carbon monoxide in significant amounts. It was found that gassed

birds had higher Gardiner a values and higher visual color scores in both raw and cooked meat. This suggests that the inhaled gases migrated to the muscle tissue where they had the ability to form hemochromes. Pool (1956) found that turkey roasted uncovered in gas ovens developed a pink color throughout the meat. The flames of gas ovens generate small amounts of nitric oxide and carbon monoxide (Pool, 1956) which can than bind to heme iron to form hemochromes.

Under the right conditions, any electronegative atom can bind to heme iron to form a complex which may produce a pink color. It then follows that contamination of a meat by substances other than nitrate and nitrite can cause the pink defect. Extensive denaturation of proteins in the meat can lead to formation of denatured protein hemochromes (Tappel, 1957). Added nicotinamide to meat can bind to the heme pigments to cause a pink color (Claus et al., 1994; Cornforth et al, 1986). Nicotinamide levels are especially high in turkey meat which has 0.083 mg/g (Richardson et al, 1980) as compared to beef which has 0.049 mg/g (Anderson, 1992) and pork with 0.039 mg/g (Anderson and Hoke, 1990). Therefore, the nicotinamide hemochrome may be a more prevalent cause of pinking in turkey. Ammonia, which is present in animal production facilities, refrigeration systems, and many sanitizers, has also been shown to cause a pink defect in cooked pork as determined by absorption spectra and increased CIE a* value (Shaw et al., 1992). The mechanism of pink formation by ammonia was not believed to be solely due to an increase in pH and the absorption spectra revealed that ammonia itself may form a pink hemochrome (Shaw et al., 1992).

2.6.5 Effect of storage time and temperature

Claus et al. (1994) reported that as the refrigerated storage time of turkey rolls increased, the CIE a* value and sensory pinkness score increased. Under the anaerobic conditions within the center of large roasts, metmyoglobin may be reduced during refrigerated storage (Claus et al., 1994). This reduction makes the formation of hemochromes with amino acids and nicotinamide more favorable (Cornforth et al., 1986). In addition, product that is chilled slower after cooking also has higher CIE a* and sensory pinkness scores (Claus et al., 1994). Ghorpade and Cornforth (1993) showed that pork roasts cooked to 82°C and which showed no initial pink color, developed a pink color with refrigerated storage. Again, reduction of the environment under refrigerated storage may be the cause of this defect. Howe et al. (1982) noted that freezing had a smaller effect on color than refrigeration and that lower temperatures may have retarded the chemical and physical reactions that favor hemochrome formation.

2.6.6 Effect of oxidation-reduction potential (ORP)

Cornforth et al. (1986) reported that the ORP is the most important variable affecting the appearance of meat. The ORP of meat determines whether the ferrous or ferric form of heme iron will predominate. The oxidation state of the iron is one of the most important factors in determining what ligand will bind to the heme and ,therefore, what color will be perceived. The reflectance spectrum of pink turkey rolls shows that reduced hemochromes of nicotinamide and globin were the predominant pigments (Cornforth et al., 1986). This means that a low oxidation-reduction potential favors the

formation of pink hemochromes. To confirm this, Cornforth et al (1986) added potassium iodate (an oxidizing agent) to the turkey rolls and the pink discoloration was inhibited.

2.7 Strategies for the Prevention of Pinking

There are several strategies which may prove effective in the prevention of pinking. First, it should be made certain that all of the native heme pigments in the meat are denatured. If present, undenatured myoglobin pigments could exist as deoxymyoglobin (dark purple) or oxymyoglobin (bright red). Near complete denaturation should be an attainable goal for myoglobin and hemoglobin but the heat stability and regeneration of cytochrome c may pose problems. Since it is unclear how much of a role cytochrome c plays in pink discoloration, extreme methods of denaturation targeted at cytochrome c should be avoided as this may affect other meat qualities.

Reduction of pinking may be possible by eliminating contamination of the meat by ligands such as nitrate, nitrite, ammonia, nitric oxide, carbon monoxide, etc. If the number of different ligands available to form heme complexes is lower, than fewer types of hemochromes will be formed.

Based on research by Cornforth et al. (1986), it may seem reasonable to add oxidizing agents to meats in order to raise the oxidation-reduction potential. While this will eliminate the pink defect, oxidizing conditions favor lipid oxidation. When lipids in meat are oxidized a distinctive off flavor is generated. In essence, this approach trades one problem for another.

Dobson and Cornforth (1992) added non-fat dry milk (NFDM) to turkey rolls in an effort to reduce pinking. Turkey rolls with 3% added NFDM showed no visible pink discoloration and had a very low redness value. It should be noted that no pinking agents (e.g. sodium nitrite or nicotinamide) were added to produce a pink color. Since the pink defect occurs sporadically, they had no way of knowing if the elimination of pinking was due to chance or the effects of the NFDM. The mechanism of NFDM's action is unclear at this time. It may simply be that the casein micelles mask the pink color or that reactive protein side chains of the NFDM raised the ORP. Another possibility is that the proteins in NFDM may denature at a lower temperature and bind with the heme ring to form a non pink complex. The NFDM may have also decreased protein denaturation in the meat so that less amino acids were available to form hemochromes (Dobson and Cornforth, 1992).

It may be possible to reduce pinking by binding a ligand to the heme that does not cause a pink color. As a consequence of the search for nitrite substitutes that will form a stable cured color, thousands of compounds were tested (Dymicky et al., 1975, Jalinski, 1971, Jalinski, 1974). From the compounds that did not result in a cured color, it may be possible to find a compound that had bound to the heme and did not cause a pink color. It may also be possible to find such a ligand among metal chelating agents that are utilized in medical therapy.

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

Brown, W.D. 1962. The concentration of myoglobin and hemoglobin in tuna flesh. J. Food Sci. 27:26-28.

Brown, W.D., Martinez, M., Johnstone, M., and Olcott, H.S. 1962. Comparitive biochemistry of myoglobins. J. Biol. Chem. 237:81-84.

Brown, W. D. and Tappel, A.L. 1957. Identification of the pink pigment of canned tuna. Food Research. 22:214-219.

Burson, D.E., Hunt, M.C., Schafer, D.E., Beckwith, D., and Garrison, J.R. 1983. Effects of stunning method and time interval from stunning to exsanguination on blood splashing in pork. J. Anim. Sci. 57:918-921.

Butt, W.D. and Keilin, D. 1962. Absorption spectra and other properites of cytochrome c and of its compounds with ligands. Proc. R. Soc. Lond. B. Biol. Sci. 156:429-458.

Claus, J.R., Shaw, D.E., and Marcy, J.A. 1994. Pink color development in turkey meat as affected by nicotinamide, cooking temperature, chilling rate, and storage time. J. Food Sci. 59:1283-1285.

Clydesdale, F.M. and Francis, F.J. 1971. The chemistry of meat color. Food Prod. Dev. 5:81,82,87-90.

Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., and Bartholomew, D.T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51:1132-1135.

Cornforth, D.P. 1989. Color-fresh, cooked, cured. Western States Meat Ass. Annual Meat Processing Conf. 41-53.

Cornish, D.G. and Froning, G.W. 1974. Isolation and purification of turkey heme proteins. Poultry Sci. 53:365-377.

Cutaia, A.J. and Ordal, Z.J. 1964. Pigment changes in anaerobically packaged ground beef. Food Tech. 18:163-166.

Dobson, B.N. and Cornforth, D.P. 1992. Nonfat dry milk inhibits pink discoloration in turkey rolls. Poultry Sci. 71:1943-1946.

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 contnet of each chromoprotein to body mass. J. Biol. Chem. 182:317-333.

Draudt, H.N. 1969. Effect of heating on the behavior of meat pigments. 22nd Proc. Rec. Meat Conf. 180-190.

Dymicky, M., Fox, J.B., and Wasserman, A.E. 1975. Color formation in cooked model and meat systems with organic and inorganic compounds. J. Food Sci. 40:306-309.

Flemming, B.K. 1990. The nature and role of hemoglobin, myoglobin, and cytochrome c in poultry meats. M.S. Thesis. University of Nebraska.

Fleming, H.P., Blumer, T.N., and Craig, H.B. 1960. Quantitative estimations of myoglobin and hemoglobin in beef muscle extracts. J. Anim. Sci. 19:1164-1177.

Fox, J.B. 1966. The chemistry of meat pigments. J. Agric. Food Chem. 14: 207-210.

Fox, J.B., Dymicky, M. and Wasserman, A.E. 1974. Heme-protein-ligand interactions. Internat. Proc. of Symp. on Protein Metal Interactions. 97-108.

Froning, G.W. and Hartung, T.E. 1967. Effect of dietary nitrates and nitrites on color of chicken meat. Poultry Sci. 46:1261.

Froning, G.W., Daddario, J., and Hartung, T.E. 1968. Color and myoglobin concentration in turkey meat as affected by age, sex, and strain. Poultry Sci. 47:1827-1135.

Froning, G.W., Mather, F.B., Daddario, J., and Hartung, T.E. 1969a. Effect of automobile exhaust fume inhalation by poultry immediately prior to slaughter on color of meat. Poultry Sci. 48:485-487.

Froning, G.W., Daddario, J., Hartung, T.E., Sullivan, T.Wand Hill, R.M. 1969b. Color of poultry meat as influenced by dietary nitrates and nitrites. Poultry Sci. 48:668-674.

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

Ghorpade, V.M., Cornforth, D.P. and Sisson, D.V. 1992. Inhibition of red discoloration in cooked vacuum packed bratwurst. J. Food Sci. 57:1053-1055.

Ghorpade, V.M. and Cornforth, D.P. 1993. Spectra of pigments responsible for pink color in pork roasts cooked to 65° or 82°C. J. Food Sci. 58:51-52, 89.

Giddings, G.G. 1977a. The basis of color in muscle foods. Crit. Rev. Food Sci. Nutr. 8: 81-114.

Giddings, G.G. 1977b. The basis of color in muscle foods. J. Food Sci. 42:288-294.

Girard, B., Vanderstoep, J., and Richards, J.F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55:1249-1254.

Hagerdal, B. and Martens, H.. 1976. Influence of water content on the stability of myoglobin to heat treatment. J. Food Sci. 41:933-937.

Hazell, T. 1982. Iron and zinc compounds in the muscle meats of beef, lamb, pork, and chicken. J. Sci. Food Agric. 33:1049-1056.

Helmke, A. and Froning, G.W. 1971. The effect of end point cooking temperature and storage on the color of turkey meat. Poultry Sci. 50:1832-1836.

Howard, A., Duffy, P., Else, K., and Brown, W.D. 1973. Possible substitutes for nitrite for pigment formation in cured meat products. J. Agric. Food Chem. 21: 894-898.

Howe, J.L., Gullett, E.A., Usborne, W.R. 1982. Development of pink color in cooked pork. Can. Inst. Food Sci. and Tech. 15:19-23.

Hunt, M.C. and Hedrick, B. 1977. Chemical, physical, and sensory characteristics of bovine muscle from four quality groups. J. Food Sci. 42:716-720.

Jalinski, T. 1971. Screening of various chemicals for cure color forming ability. Oscar Mayer.

Jalinski, T. 1974. Cure color forming ability of several nitrogen containing compounds. Oscar Mayer.

Janky, D.M. and Froning, G.W. 1973. The effect of pH and certain additives on heat denaturation of turkey meat myoglobin. Poultry Sci. 52:152-159.

Kagen, L.J. 1973. *Myoglobin: Biochemical, Physiological, and Clinical Aspects.* Columbia University Press. New York, NY.

Keilin, D. 1966. *The History of Cell Respiration and Cytochrome.* Cambridge at the University Press. p.316-353.

Keilin, J. 1960. Nature of the haem-binding groups in native and denatured haemoglobin and myoglobin. Nature 187:365-371.

Kinsman, D.M., Kotula, A.W., and Breidenstein, B.C. 1994. *Muscle Foods-Meat poultry, and seafood technology.* Chapman and Hall Inc. New York, NY.

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

Lawrie, R.A. 1991. *Meat Science*, Fifth Edition. Pergamon Press. New York, NY.

Ledward, D.A., and Shorthose, W.R. 1971. A note on the haem pigment concentration of lamb as influenced by age and sex. Anim. Prod. 13:193-195.

Lehninger, A.L., Nelson, D.L., and Cox, M.M. 1993. *Principles of Biochemistry*. Second Edition. Worth Publishers. New York, NY.

Livingston, D.J. and Brown, W. D. 1981. The chemistry of myoglobin and its reactions. Food Tech. 35:244-252.

Margoliash, E. and Bosshard, H.R. 1983. Guided Electrostatics, a textbook protein comes of age. Trends Biochem. Sci. 8:316-320.

Moore, G.R. and Pettigrew, G.W. 1990. *Cytochromes c-Evolutionary, structural, and physiochemical aspects*. Springer-Verlag. Berlin Heidelberg.

Mugler, D.J., Mitchell, J.D., and Adams, A.W. 1970. Factors affecting turkey meat color. Poultry Sci. 48:1510-1513.

Nash, D.M., Proudfoot, F.G., Hulan, H.W. 1985. Pink discoloration in cooked broiler chicken. Poultry Sci. 64:917-919.

Ngoka, D.A., Froning, G.W., Lowry, S.R., and Babji, A.S. 1982. Effects of sex, age, preslaughter factors, and holding conditions on the quality characteristics and chemical composition of turkey breast muscles. Poultry Sci. 61:1996-2003.

Ngoka, D.A. and Froning, G.W. 1982. Effect of free struggle and preslaughter excitement on color of turkey breast muscles. Poultry Sci. 61:2291-2293.

Niewiarowicz, A.., Pikul, J., and Czajka, P. 1986. Gehalt an myoglobin und hamoglobin in fleisch verschiedener geflugelarten. Fleischwirtschaft 66:1281-1282.

Nishida, J. 1976. Changes in myoglobin content during development and growth of chicken. Jap. J. Vet. Sci. 38:299.

Pikul, J., Niewiarowicz, A., and Kupijaj, H. 1986. The cytochrome c content of various poultry meats. J. Sci. Food Agric. 37:1236-1240.

Price, J.F. and Schweigert, B.S. 1971. *The Science of Meat and Meat Products*, Second Edition. W.H. Freeman and Company. San Francisco, CA.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. 31:68,69,72-44.

Richardson, M., Posati, L.P., and Anderson, B.A. 1980. Composition of foods. Sausage and luncheon meats (Raw, Processed, Prepared). Handbook No. 8-7. United States Department of Agriculture, Science and Education Administration. Washington D.C.

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

Shaw, D.E., Claus, J.R., and Stewart, K.K. 1992. Effects of ammonia exposure on fresh pork: a distinct pink color after cooking. J. Muscle Foods 3:169-174.

Solberg, M. 1970. The chemistry of color stability in meat - A review. Can. Inst. Food Tech. 3:55-62.

Tappel, A.L. 1957. Reflectance spectral studies of the hematin pigments of cooked beef. Food Research. 22: 404-407.

Topel, D.G., Merkel, R.A., Mackintosh, D.L., and Hall, J.L. 1966. Variation of some physical and biochemical properties within and among selected porcine muscles. J. Anim. Sci. 25:277-282.

Trout, G.R. 1989. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. J. Food Sci. 54:536-540.

Van De Graaff, K.M., and Fox, S.I. 1989. *Concepts of Human Anatomy and Physiology.* Second Edition. Wm. C. Brown Publishers. Dubuque, IA.

Van Gelder, F.F. and Slater, E.C. 1962. The extinction coefficient of cytochrome c. Biochem. Biophys. Acta. 58:593-595.

Walsh, K.A. and Rose, D. 1956. Factors affecting the oxidation of nitric oxide myoglobin. J. Agric. Food Chem. 4:352-355.

Warriss, P.D. 1977. The residual blood content of meat-A Review. J. Sci. Food Agric. 28:457-462

Warriss, P.D. and Rhodes, D.N. 1977. Haemoglobin concentrations in beef. J. Sci. Food Agric. 28:931-934.

Warriss, P.D. and Leach, T.M. 1978. The influence of slaughter method on the residual blood content of meat. J. Sci. Food Agric. 29:608-610.

Chapter 3

Quantification of the Nicotinamide Hemochrome Pigment in Cooked, Uncured Turkey by Reflectance Spectrophotometry

3.1 Abstract

Nicotinamide hemochrome has been reported as one of the potential pigments involved in the pinking defect of cooked, uncured turkey. Reflectance spectroscopy has been the principle method used to identify this pigment. However, a reliable, easy method to quantify its presence has not been developed. Cooked turkey samples (containing 0, 0.25, 0.50, 1.0, or 2.0% nicotinamide or 0, 75, or 150 ppm sodium nitrite) were treated with potassium ferricyanide, hydrogen peroxide, sodium dithionite and intense light. Treated samples were analyzed with a reflectance spectrophotometer in the visible range. From analysis of reflectance wavelength ratios of the nicotinamide graphs and correlations of those values, it was determined that the ratio of 537/553 nm best represented the relative quantity of nicotinamide hemochrome (r=0.993) while poorly representing nitrosylhemochrome (r=0.029).

3.2 Introduction

A pink color defect has been observed on the fresh cut surfaces of cooked, uncured turkey rolls. When the study of this phenomenon began, it was believed that formation of nitrosylhemochrome, which resulted from contamination of nitrate, nitrite, or nitric oxide, was the mechanism responsible (Cornforth et al., 1986). This contamination

was hypothesized to originate from the diet of the animals (Froning and Hartung, 1967), processing equipment, carcass chill water (Mugler et al., 1970), exhaust fumes of transportation vehicles (Froning et al., 1969), and gas cooking ovens (Pool, 1956).

Recent studies have questioned if nitrosylhemochrome was really the pigment responsible for the pink defect in turkey. Cornforth et al. (1986) attempted to identify the pigment responsible for the pink defect in uncured turkey rolls. Commercial pink samples were analyzed and no residual nitrite or nitrosylhemochrome were found. Hornsey (1956) determined that nitrosylhemochrome was extractable with an acetone-water solvent. The pink pigment in commercial samples studied by Cornforth et al. (1986) could not be extracted in water, acetone or other solvents. From these findings they concluded that the pink defect was not due to nitrite contamination.

An alternative explanation for the pink defect could be that the pigment was another hemochrome which was formed with a different nitrogenous ligand. Akoyunoglou et al. (1963) characterized hemochromes as having a typical dual absorption spectrum with maxima at 520-530 nm and 555-565 nm. Cornforth et al. (1986) found that cooked turkey with 2.0% added nicotinamide had a reflectance spectrum similar to a commercial pink turkey sample. Those two samples also exhibited the typical hemochrome absorption spectrum. Brown and Tappel (1957) observed a pink color in canned tuna and determined that the primary pigment was a mix of nicotinamide and denatured-globin hemochromes. The high content of nicotinamide (0.083 mg/g) in turkey meat (Richardson et al., 1980) favors the formation of reduced nicotinamide-denatured-globin hemochromes.

It seems likely that hemochromes, especially nicotinamide hemochromes, are at least partially responsible for the pink defect. The principal way to identify the nicotinamide hemochrome is by reflectance and absorbance spectroscopy and by their resistance to extraction by acetone and water. However, there is not an easy method to quantify the presence of the nicotinamide hemochrome. Nitrosylhemochrome quantity can be determined (Kraft and Ayres, 1954) by dividing the percent reflectance at 570 nm by the percent reflectance at 650 nm (%R570 nm/%R650 nm). Other pigments like deoxymyoglobin, oxymyoglobin, and metmyoglobin can be quantified in a similar fashion. The objective of this study was to develop a method of reflectance-wavelength manipulation to quantify nicotinamide hemochrome.

3.3 Materials and Methods

3.3.1 Preparation of Turkey

Turkey *Pectoralis major* muscles (3 days old) obtained from a local Virginia processor were ground twice through a 4.8 mm plate (model 4532, Hobart® Manufacturing Co., Troy, Ohio). For each treatment 100 g of turkey were weighed out and 2.0 g of sodium chloride and 0.5 g of sodium tripolyphosphate were added. Treatments consisted of a control (distilled, deionized water only), two levels of sodium nitrite (75 and 150 ppm) and four levels of nicotinamide (0.25, 0.50, 1.0, and 2.0%). The stock solutions of sodium nitrite and nicotinamide were 1.5 mg/ml and 100 mg/ml, respectively. As needed, each treatment volume was brought to 20 ml with distilled water (20% pump on a meat weight basis). Meat and ingredients were mixed at low speed for

approximately one min using a hand mixer (model KHM3WH-1, Kitchen Aid®, St. Joseph, Michigan). For each treatment, two centrifuge tubes (50 ml 28x15 mm polypropylene tubes with screw plug seal, #05-539-9, Fisher Scientific, Pittsburgh, Pennsylvania) were stuffed with approximately 45 g of the ground meat mix and capped. Tubes were placed in a low speed centrifuge (model 54343, The Jalco Co., Union City, Indiana) for approximately five min at the highest speed setting to pack the meat and eliminate air pockets. Next, the covered tubes were placed in an 85°C water bath (model 10 L, Fisher Scientific Co., Pittsburgh, Pennsylvania). The temperature of the samples and the water bath were monitored by thermocouples attached to a data logger (model 5100, Electronic Controls Design, Milwaukee, Oregon). Meat was cooked to an internal temperature of 80°C and placed in refrigerated storage (2-4°C) overnight. Three complete replications of each treatment were manufactured.

3.3.2 Sample Treatments and Analysis

Modified procedures of Erdman and Watts (1957) and Cornforth et al. (1986) were used in order to manipulate the muscle pigments. Stock solutions of potassium ferricyanide (PF, 0.1%), hydrogen peroxide (HP, 0.05%), sodium dithionite (SD, 0.1M) were prepared and approximately 60 ml of each were placed in covered containers. Cooked samples were individually removed from the tubes (2 tubes per treatment) and sliced longitudinally into two halves. Sample halves were incubated in each of the three solutions (4 h PF, 20 min HP, 10 min SD) at room temperature in a covered (dark storage), styrofoam cooler. Instead of incubation in a solution, another half was analyzed immediately after cutting (fresh surface) and after light fading as a result of a 2 min of

exposure to 107 lux of fluorescent light (model L096080, Extech Instrument Corp., Waltham, Mass). Analysis of each sample was performed with a UV-visible scanning spectrophotometer (model 2101PC, Shimadzu Corporation., Kyoto, Japan) to obtain reflectance readings from 400-700 nm. The spectrophotometer was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm, and fast scan speed. Calibration was performed using a Minolta white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44).

3.3.3 Manipulation of Reflectance Data

Means of the three replications were taken so that data for the control, nitrite, and nicotinamide samples could be plotted (percentage reflectance verses wavelength) such that each graph contained a fresh, faded, H_2O_2, oxidized, and reduced curve. Wavelength ranges were then chosen from the nicotinamide curves and manipulated to provide percentage reflectance ratios and differences.

3.3.4 Statistical Analysis

The percentage reflectance ratios and differences for all four nicotinamide samples (0.25, 0.50, 1.0, 2.0%) were averaged and analyzed using the correlation coefficient procedure of SAS (1989). The same procedure was used to analyze both sodium nitrite levels (75, 150 ppm).

3.4 Results and Discussion

Samples were chemically treated to determine unique reflectance peaks and valleys for nicotinamide hemochrome. The analysis immediately after cutting was to obtain a

fresh, non-light faded pigment measurement. The 2 min light exposure was to obtain and measure the faded pigment. SD (a reducing agent) was used to insure a reduced pigment (Cornforth et al., 1986) and PF (an oxidizing agent) was used to obtain an oxidized pigment (Erdman and Watts, 1957). Erdman and Watts (1957) determined that hydrogen peroxide breaks the heme ring and renders the pigments unable to produce color.

The data for the control, nitrite, and nicotinamide samples were plotted (reflectance verses wavelength) so that there were five lines on each graph (fresh, faded, H_2O_2, oxidized, and reduced). The control sample graph (Figure 1) had very similar curves for the fresh and faded samples with a trough reflectance valley near 550 nm. Reducing (SD) the control made the trough more shallow and shifted it to the right (560 nm). Oxidizing (PF) the sample removed all significant peaks and valleys in the 500-600 nm range. Treatment with hydrogen peroxide had an effect similar to PF.

The nicotinamide treated samples, regardless of level, all had similar shaped reflectance curves. Figure 2 depicts the 2.0% nicotinamide treated samples. A reflectance valley at approximately 555 nm and a peak at approximately 533 nm were present for the fresh, faded, and reduced samples. In addition, the trough was most shallow for the faded sample (~ 43% reflectance), followed by the fresh samples (~ 37% reflectance), and was deepest for the reduced sample (~ 35% reflectance). As with the control sample, the troughs and peaks disappeared in the oxidized sample and the hydrogen peroxide sample curve was essentially flat.

As the level of nicotinamide in the samples increased (0.25, 0.50, 1.0, 2.0%), the reflectance values at 533 nm and 555 nm decreased (Figure 4). The peak at 533 nm

decreased; 50%, 49%, 47%, 45%, respectively. Over the same nicotinamide levels, the reflectance valley at 555 nm decreased; 48%, 46%, 42%, 37% reflectance, respectively.

The sodium nitrite graphs were similar for the two levels but very different than the control and nicotinamide graphs. Figure 3 illustrates the 75 ppm sodium nitrite curves. A valley was near 575 nm and a peak was near 510 nm for the fresh, faded, and reduced curves. The oxidized curve was shifted upward and had a valley near 535 nm and a peak near 500 nm. Hydrogen peroxide treatment did not greatly alter the curve from the typical nitrite spectrum. This may be because nitrite stabilizes the heme ring, making it more resistant to breakage from hydrogen peroxide at 0.05%.

Based on the nicotinamide graphs (Figure 2), wavelength ranges were chosen +/- 3 nm from the major inflection. It was crucial that the chosen ranges did not have a similar graph trend as the nitrite samples. For example, if a point of inflection occurred at the same wavelength on both the nicotinamide and nitrite curves, that wavelength was omitted from consideration. The ranges chosen were valleys (552-558 nm and 521-527 nm) and peaks (533-539 nm and 477-483 nm). All possible ratio and difference combinations (from the fresh and reduced curves) were made between ranges that were adjacent to each other on the reflectance curves (552-558 with 533-539, 533-539 with 521-527, 521-527 with 477-483). Identical wavelength ranges were chosen for the fresh and reduced curves from the nitrite graph (Figure 3) and the same ratio and difference comparisons were made. The ratios and differences were then correlated. A ratio or difference with a high correlation for nicotinamide samples and a low correlation for nitrite samples was desired so that the predictor for nicotinamide hemochrome was not also a

good predictor of nitrosylhemochrome. The ratios and differences for the 521-527 and 477-483 set were screened out because the nitrite correlations were very high. The range comparison of 533-539 divided by 552-558 produced the best correlations (Table 1). The highest correlation for fresh nicotinamide samples with the lowest fresh nitrite sample correlation was obtained by the ratio of 537/553 nm. The ratio of 537/553 also had the best correlation (r = 0.782) with the reduced nicotinamide sample. These particular wavelengths also do not correspond to distinct peaks or valleys in the fresh meat pigments (deoxymyoglobin, oxymyoglobin, metmyoglobin). Because this was a ratio of a larger number divided by a smaller number (when nicotinamide hemochrome is present), the value obtained was greater than one. As the quantity of nicotinamide hemochrome increases, the ratio became larger.

3.5 Conclusions

The development of an easy method to quantify the presence of nicotinamide hemochrome becomes necessary in light of the potential role of this pigment in the pinking defect of cooked, uncured turkey. With the ability to quantitate this pigment, it is now possible to more accurately determine the effects of various pre-harvesting technologies, postmortem processing, and non-meat ingredients on the occurrence of the nicotinamide hemochrome pigment. Reflectance spectroscopy has been one of the primary methods for identifying the presence of nicotinamide hemochromes and extending that methodology to quantify this pigment was logical. Through the correlation of reflectance wavelength ratios and differences of nicotinamide treated samples (and the lack of correlation of nitrite

treated samples) the ratio of %R537 nm/%R553 nm has been found to represent the relative level of nicotinamide hemochrome.

3.6 References

Akoyunoglou, J-H.A., Olcott, H.S., and Brown, W.D. 1963. Ferrihemochrome and ferrohemochrome formation with amino acids, amino acid esters, pyridine derivatives and related compounds. Biochem. 2:1033-1041.

Brown, W. D. and Tappel, A.L. 1957. Identification of the pink pigment of canned tuna. Food Research. 22:214-219.

Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., and Bartholomew, D.T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51:1132-1135.

Erdman, A.M. and Watts, B.M. 1957. Spectrophotometric determination of color change in cured meat. J. Agric. Food Chem. 5:453-455.

Froning, G.W. and Hartung, T.E. 1967. Effect of dietary nitrates and nitrites on color of chicken meat. Poultry Sci. 46:1261.

Froning, G.W., Mather, F.B., Daddario, J., and Hartung, T.E. 1969. Effect of automobile exhaust fume inhalation by poultry immediately prior to slaughter on color of meat. Poultry Sci. 48:485-487.

Hornsey, H.C. 1956. The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 7:534-540.

Kraft, A.A. and Ayres, J.C. 1954. Color changes in packaged bologna. Food Tech. 8: 22-26.

Mugler, D.J., Mitchell, J.D., and Adams, A.W. 1970. Factors affecting turkey meat color. Poultry Sci. 48:1510-1513.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. 31:68,69,72-44.

Richardson, M., Posati, L.P., and Anderson, B.A. 1980. Composition of foods. Sausage and luncheon meats (Raw, Processed, Prepared). Handbook No. 8-7. United States Department of Agriculture, Science and Education Administration. Washington D.C.

SAS, 1989. Statistical Analysis System Institute Inc., Cary, NC.

Table 1- Correlation coefficients[1] (r) for the ratios of 533-539 nm and 552-558 nm wavelength ranges for nicotinamide and sodium nitrite containing turkey samples

	Correlation Coefficient				
Ratio	Nicotinamide[2]	Sodium Nitrite[3]	Ratio	Nicotinamide[2]	Sodium Nitrite[3]
539/558	0.931***	0.827**	535/555	0.935***	0.808**
538/558	0.930***	0.800**	534/555	0.934***	0.728*
537/558	0.928***	0.783*	533/555	0.926***	0.716*
536/558	0.927***	0.823**			
535/558	0.931***	0.826**	539/554	0.935***	0.765*
534/558	0.929***	0.767*	538/554	0.935***	0.706*
533/558	0.920***	0.765*	537/554	0.933***	0.722*
			536/554	0.931***	0.775*
539/557	0.935***	0.821**	535/554	0.935***	0.739*
538/557	0.935***	0.795*	534/554	0.935***	0.646
537/557	0.932***	0.770*	533/554	0.926***	0.621
536/557	0.932***	0.818**			
535/557	0.934***	0.817**	539/553	0.935***	0.357
534/557	0.934***	0.754*	538/553	0.935***	0.109
533/557	0.926***	0.753*	537/553	0.933***	0.029
			536/553	0.930***	0.136
539/556	0.936***	0.827**	535/553	0.934***	0.125
538/556	0.936***	0.806**	534/553	0.933***	-0.069
537/556	0.934***	0.771*	533/553	0.925***	-0.061
536/556	0.933***	0.821**			
535/556	0.937***	0.833**	539/552	0.933***	-0.595
534/556	0.936***	0.766*	538/552	0.933***	-0.630
533/556	0.928***	0.760*	537/552	0.931***	-0.754*
			536/552	0.928***	-0.715*
539/555	0.934***	0.803**	535/552	0.932***	-0.640
538/555	0.934***	0.776*	534/552	0.931***	-0.797*
537/555	0.932***	0.751*	533/552	0.921***	-0.792*
536/555	0.931***	0.803**			

[1] Correlation coefficients- * P<0.05, ** P<0.01, *** P<0.001

[2] Nicotinamide correlations developed from turkey samples containing added nicotinamide (0.25, 0.50, 1.0, 2.0%)

[3] Nitrite correlations developed from turkey samples containing added sodium nitrite (75, 150 ppm)

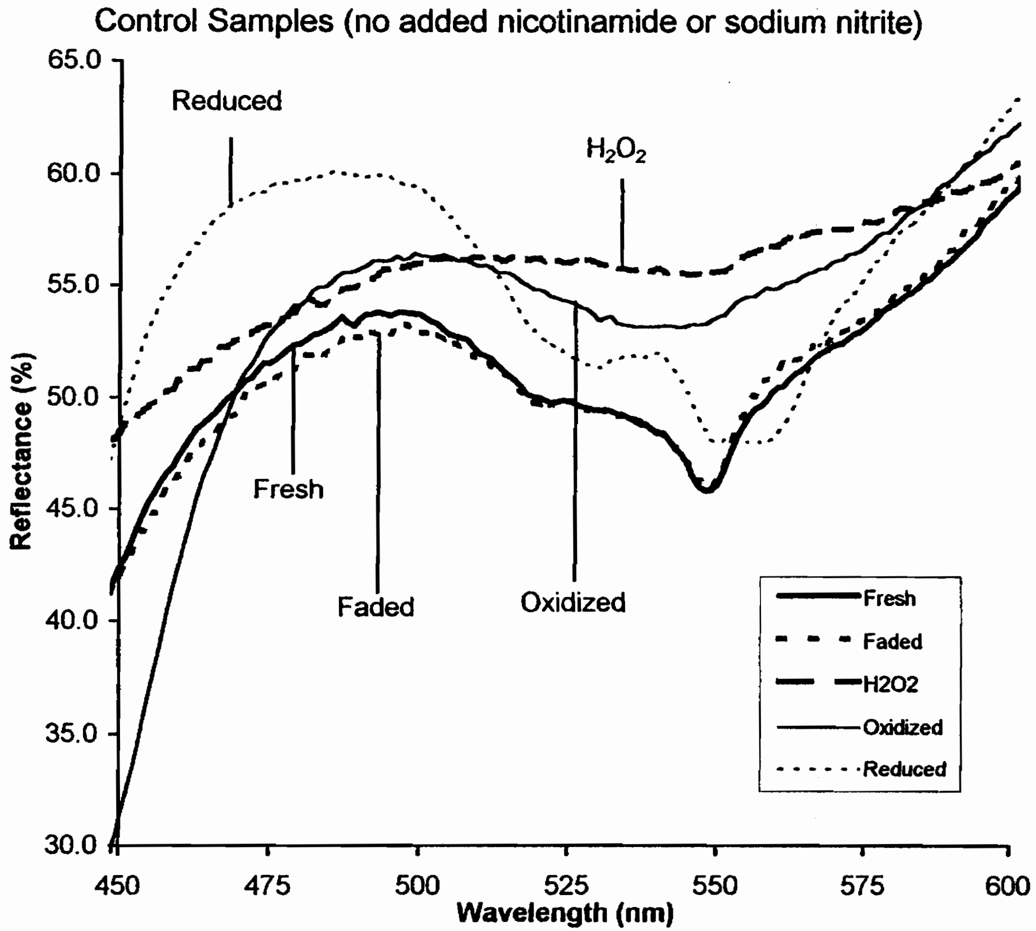


Figure 1- Reflectance versus wavelength for control samples treated with nothing (fresh), light (faded), hydrogen peroxide (H_2O_2), potassium ferricyanide (oxidized), and sodium dithionite (reduced).

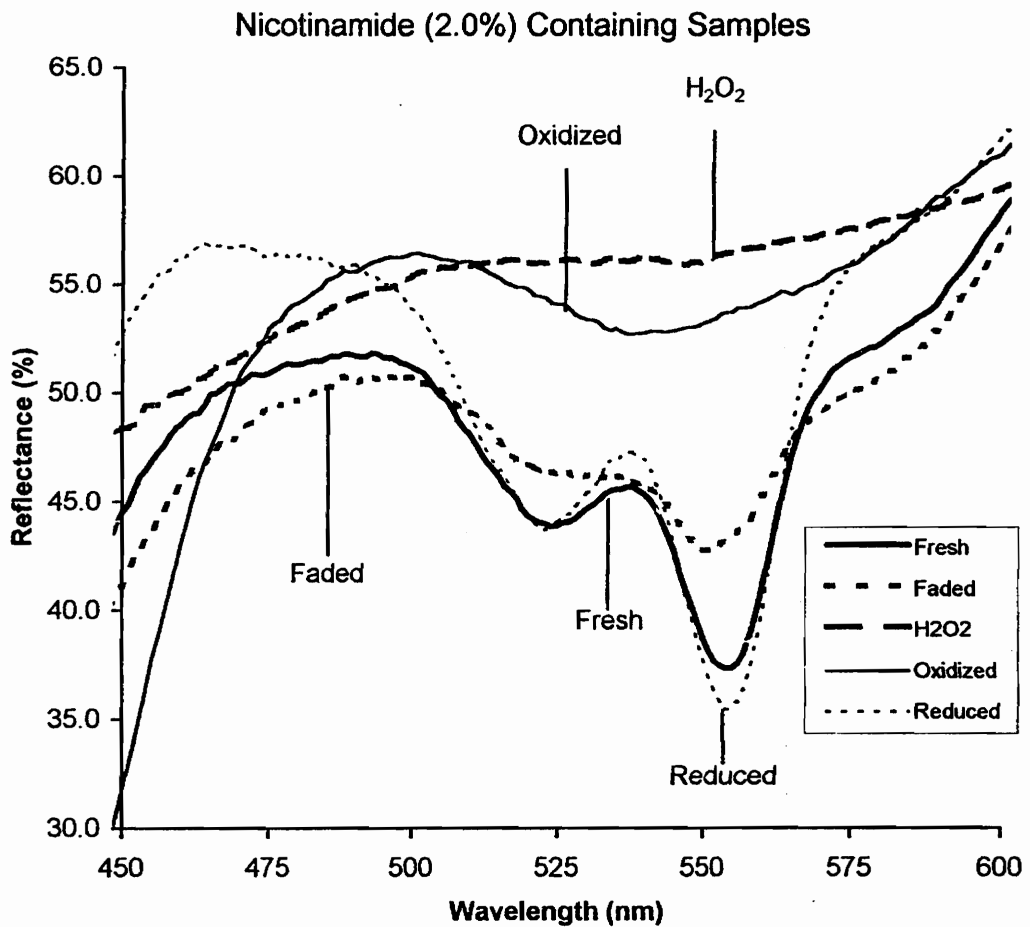


Figure 2- Reflectance versus wavelength for 2.0% nicotinamide samples treated with nothing (fresh), light (faded), hydrogen peroxide (H_2O_2), potassium ferricyanide (oxidized), and sodium dithionite (reduced).

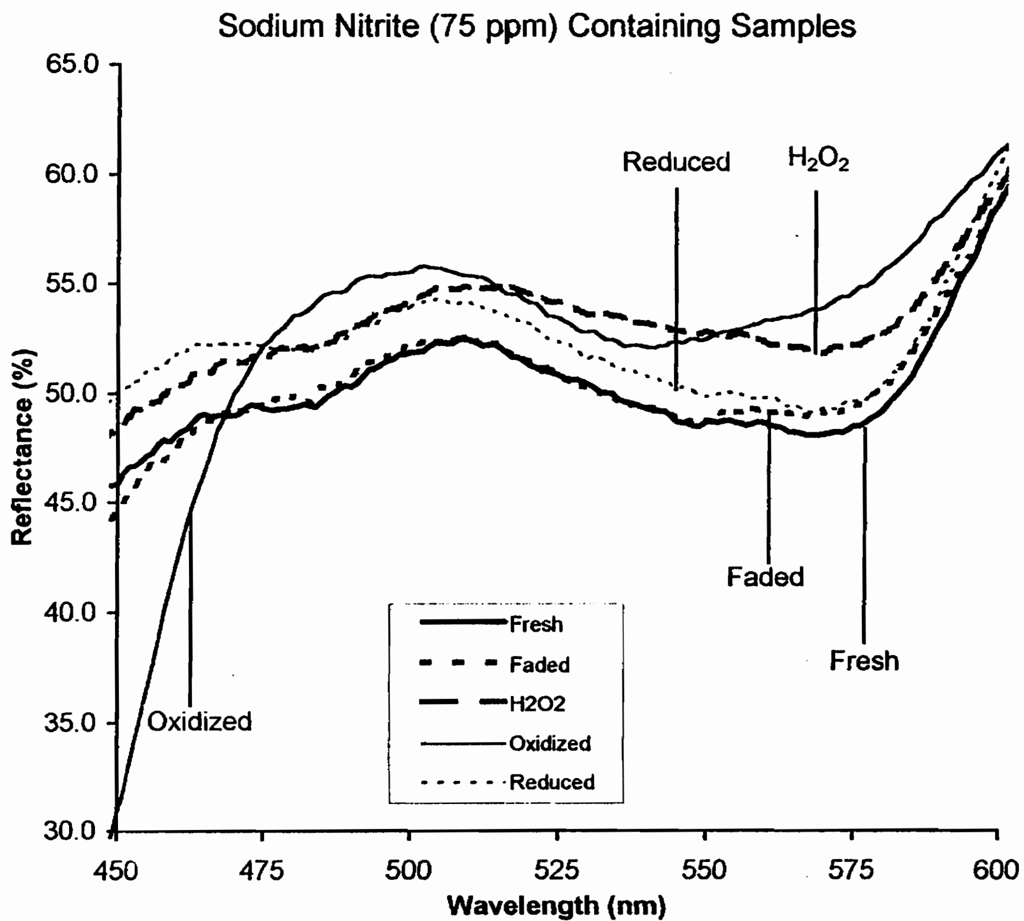


Figure 3- Reflectance versus wavelength for 75 ppm sodium nitrite samples treated with nothing (fresh), light (faded), hydrogen peroxide (H_2O_2), potassium ferricyanide (oxidized), and sodium dithoinite (reduced).

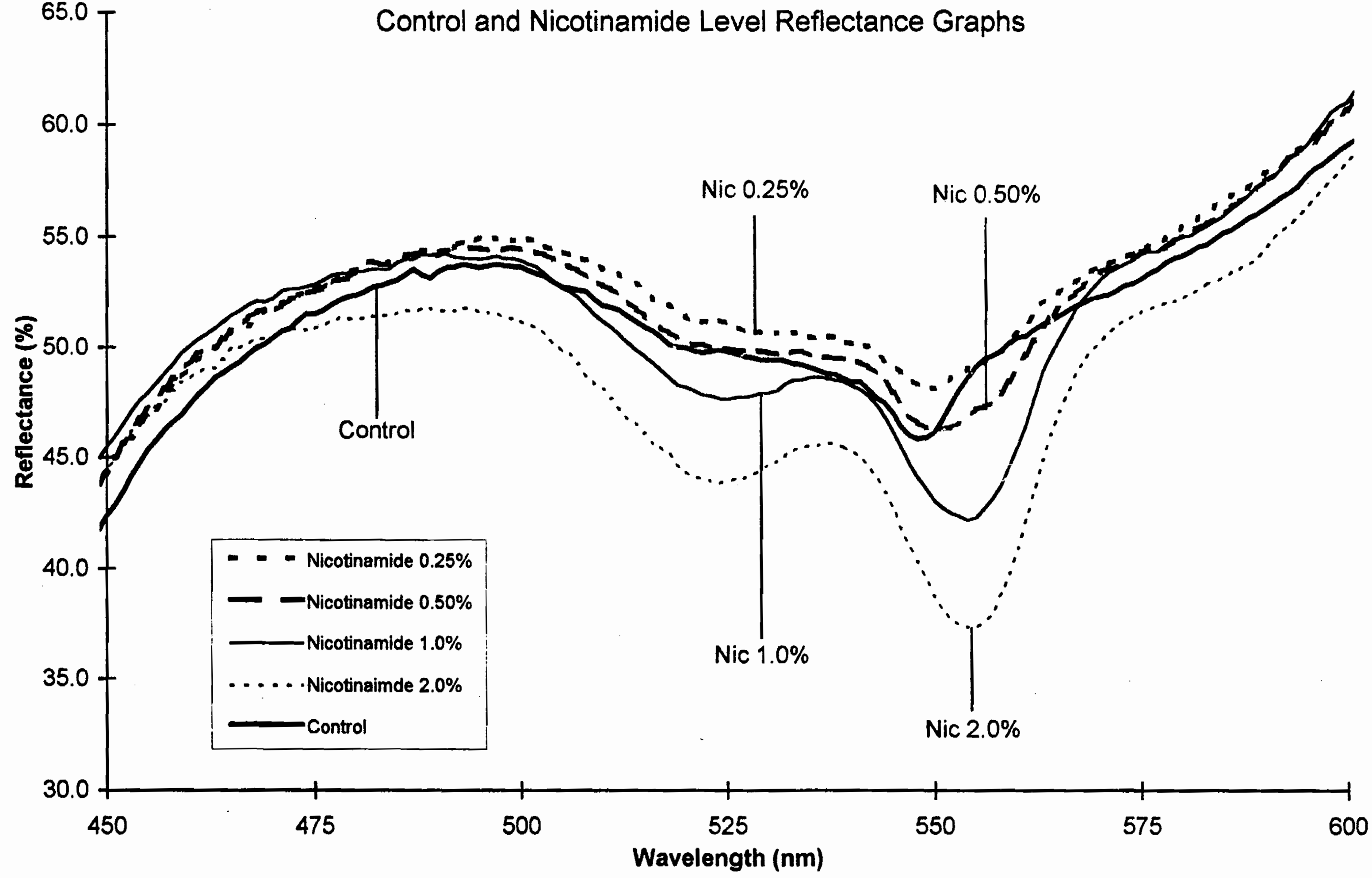


Figure 4- Control and nicotinamide level (0.25, 0.50, 1.0, 2.0%) relectance graphs

Chapter 4

Inhibition of Pinking in Cooked, Uncured Ground Turkey Through the Binding of Non-Pinking Ligands to Muscle Pigments

4.1 Abstract

The pink color defect in cooked, uncured turkey is a sporadic problem which can result in economic loss and consumer dissatisfaction. Fourteen ligands were tested in ground turkey samples for their ability to reduce pinking in control samples and in the presence of 150 ppm sodium nitrite or 1.0% nicotinamide (pinking agents). Trans 1,2-diaminocyclohexane-N,N,N',N' tetraacetic acid monohydrate (CA); diethylenetriamine pentaacetic acid (DA), ethylenedinitrilo-tetraacetic acid disodium salt (EA), and calcium reduced non-fat dry milk (NM) were the most effective at reducing pinking with and without pinking agents.

4.2 Introduction

There are many characteristics that consumers evaluate when making the decision to purchase meats. The most utilized characteristic is color because it is the easiest quality trait to evaluate. A major problem that has plagued the poultry industry, and other meat industries to a lesser degree, is a pink color defect. The pinking defect, also known as pink spot and pink ring, manifests itself as a pink coloring of the fresh cut surface of meat products (Cornforth et al., 1986) such as cooked, uncured turkey and pork rolls. The pink pigment fades rapidly upon exposure to air and its occurrence is unpredictable. Because

the pink color makes products appear undercooked, consumer complaints and commercial buyer discounting have been associated with products portraying the defect.

A wide variety of factors have been suggested as causes of pinking. Pool (1956) found that carbon monoxide and nitric oxide generated by the flames of gas ovens can combine with myoglobin to form a pink color. Likewise, exhaust fumes from automobiles (carbon monoxide and nitric oxide) inhaled by poultry in route to processing facilities have been reported to cause a pink color (Froning et al., 1969b). Nitrate and nitrite contamination during raising or processing of birds has been a widely used explanation for pinking. Nitrates and nitrites in the feed (Froning and Hartung, 1967) or drinking water (Froning et al., 1969a) of poultry may lead to a pink end product. Nitrogenous contamination can also originate in the water utilized for carcass chilling (Mugler, et al., 1970) and on processing equipment previously used for cured products. Shaw et al. (1992) found that ammonia contamination caused a pink defect in pork.

It has been demonstrated that hemochromes can form by the interaction of nitrogenous ligands other than nitrates and nitrites. Certain amino acids (Akoyunoglou et al. 1963; Ahn and Maurer, 1990), pyridine, and nicotinamide (Ahn and Maurer, 1990) are just a few of the possible ligands that may form hemochromes with meat pigments. Akoyunoglou et al. (1963) characterized hemochromes as having a typical dual absorption spectrum with maxima at 520-530 nm and 555-565 nm. Cornforth et al. (1986) found that cooked turkey with 2.0% added nicotinamide had a reflectance curve similar to a commercial pink turkey sample and it also exhibited the typical hemochrome absorption curve. Brown and Tappel (1957) observed a pink color in canned tuna and determined

that the primary pigment was a mixed nicotinamide-denatured-globin hemochrome. The high nicotinamide content of turkey meat (0.083 mg/g; Richardson et al., 1980) compared to other species favors the formation of nicotinamide hemochromes. Denatured proteins and free amino acids generated during the cooking process may also contribute to pinking through hemochrome formation.

Regardless of which native pigments or hemochromes are involved in the pinking defect, the means to control this problem has not been reported. Since hemochromes are formed when specific ligands bind the heme pigments, it may be possible to bind a ligand to the heme ring that does not cause a pink color. Ligands with pinking reduction potential may exist among compounds that were unsuccessfully tested as nitrite substitutes and among metal chelators used in medical therapy. Thus, the objective of this study was to test various ligands in ground turkey for their ability to reduce pinking in control samples in addition to pinking agent (nicotinamide and sodium nitrite) treated samples.

4.3 Materials and Methods

4.3.1 Preparation of meat mix

Whole right lobes of turkey *Pectoralis major* muscles (vacuum packaged and frozen for 2-10 months) were defrosted at 2°C two days before use. The muscles were cut into strips and ground through a 4.8 mm plate (model 4532, Hobart® Manufacturing Co., Troy, Ohio). After grinding, the meat was hand mixed and ground through a 4.8 cm plate again. The ground turkey was refrigerated at 2°C and stored for 1-3 days.

The screening process consisted of several treatments (Table 1). Stock solutions for sodium nitrite (3 mg/ml), nicotinamide (200 mg/ml), and each ligand (4 mg/ml) were formulated to 100 ml in volumetric flasks. The fourteen ligands evaluated were: 3-amino pyridine (AP); 4-benzoylpyridine (BP); trans 1,2-diaminocyclohexane-N,N,N',N' tetraacetic acid monohydrate (CA); diethylenetriamine pentaacetic acid (DA), ethylenedinitrilo-tetraacetic acid disodium salt (EA), 2,3 dihydroxybenzoic acid (DB);

Table 1- Treatment specifications

Treatment	Treatment Level (meat basis mg/g)	Meat Weight (g)	Stock Solution[1] Volume (ml)	Distilled Water volume (ml)
control	no treatment	200	0	0
sodium nitrite	0.15	200	10	10
nicotinamide	10.0	200	10	10
ligand	0.20 or 30.0[2]	200	10	10
ligand + sodium nitrite	0.20 or 30.0 0.15	200	10 10	0
ligand + nicotinamide	0.20 or 30.0 10.0	200	10 10	0

[1] Stock solutions volume- each ligand as well as sodium nitrite and nicotinamide were added to the meat from a stock solution

[2] NFDM added at 30.0 mg/g; all other ligands added at 0.20 mg/g

3-ethyl pyridine (EP); calcium reduced non-fat dried milk (NM); 2,3 phthalic acid (PA); 3-picoline (PC); pyrrole (PY); pyridazine (PZ); pyridinedicarboxcylic acid (YA); and pyrazinedicarboxcylic acid (ZA). The concentration of each stock solution was such that 10 ml of stock would yield the concentration, on a meat basis, of the treatment specified in Table 1. Every treatment contained 2.0% sodium chloride (NaCl) and 0.5% sodium

tripolyphosphate (STP) on a meat weight basis. Once the stock solutions were prepared 200 g of the ground turkey breast were weighed into a stainless steel bowl and 4.0 g NaCl (2.0%) and 1.0 g STP (0.5%) were added. A treatment was then added to the meat from a stock solution so that a volume equaling a 10% pump (20 ml per 200 g meat) was achieved. Since the concentrations of the stocks were such that 10 ml gave the proper treatment for 200 g meat, 10 ml of distilled water was added to 10 ml of stock when only one stock was used (ex. sodium nitrite treatment). When two stocks were used (ex. sodium nitrite + ligand) 10 ml of each stock was used to give a final added volume of 20 ml. For the control treatment 20 ml of distilled water was added to the meat, salt, and phosphate. Next, the meat and its ingredients were mixed at low speed for approximately one min. using a hand mixer (model KHM3WH-1, Kitchen Aid®, St. Joseph, Michigan).

4.3.2 Processing of the meat mix

Once prepared, 45 g of the mixture was loosely stuffed into centrifuge tubes (50 ml 28x15 mm polypropylene tubes with screw plug seal, #05-539-9, Fisher Scientific Co., Pittsburgh, Pennsylvania). Three tubes (repeated measures) for each treatment were prepared for every replication (three). In order to compact the meat and reduce void spaces, tubes were capped and placed in a low speed centrifuge (model 54343, Jalco Motor Company, Union City, Indiana) for approximately five min. at the highest speed setting. After centrifugation, the covered tubes were placed in an 85°C water bath (model 10L, Fisher Scientific Co., Pittsburgh, Pennsylvania). The temperature of the samples was monitored by thermocouples attached to a data logger (model 5100, Electronic Controls

Design, Milwaukee, Oregon). The meat was cooked to an internal temperature of 80°C and placed in refrigerated storage (2°C) overnight.

4.3.3 Characterization of color

Meat plugs were individually removed from the tubes and a cross sectional slice was made approximately 2 cm from the top of the plug. Immediately after cutting, CIE L* a* b* readings were measured with a chroma meter (model CR-200, Minolta Corp., Osaka, Japan) using a 1 cm aperture placed at a right angle to the sample. The device was calibrated using a standard Minolta white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44, part # 20933026, Minolta Corp., Osaka, Japan). One reading was taken on each of the three repeated measures and the values were averaged using the statistic function of the chroma meter.

The remaining samples were sliced longitudinally and reflectance readings (400-700 nm) were taken using a UV-visible-scanning spectrophotometer (model 2101PC, Shimadzu Inc., Kyoto, Japan). The spectrophotometer was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm, and fast scan speed. Calibration was performed using the Minolta white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44, part # 20933026, Minolta Corp., Osaka, Japan).

4.3.4 Statistical analysis

Dependent variables were analyzed using the General Linear Model (GLM) procedure of SAS® (1989) in a randomized block design (15 treatments x 3 replications).

If the models contained significant differences, mean values were separated using the Least Significant Difference procedure of SAS. Set one analyzed the control and the 14 ligands. Set two analyzed the sodium nitrite treatment and the 14 ligand plus sodium nitrite treatments. Set three analyzed the nicotinamide treatment and the 14 ligand plus nicotinamide treatments.

4.4 Results and Discussion

4.4.1 Effect of Ligands Without Added Pinking Agents

During the first stage of this experiment each ligand was evaluated in a ground turkey system without added pinking agents. This set of conditions approximated normal commercial product color because nothing was added specifically to produce a pink color. Therefore, any pink color that was present and reduced by a ligand was the "natural", spontaneous pink. This 'ligand only' testing also served to insure that the ligands themselves did not produce a pink color. The ligand samples were compared to the control to evaluate their specific effects.

CIE L*a*b*

Many of the ligands had significant effects on the CIE L*a*b* measurements (Table 2). The control had an L* value of 75.29. BP was the only ligand to reduce ($P<0.05$) this value while CA and DA were the only ligands that made the samples lighter ($P<0.05$) than the control. The a* value (4.21 control) was lowered ($P<0.05$) by CA, DA, EA, NM, and PZ. A decrease in the CIE a* value was demonstrated by Dobson and Cornforth (1992) from the addition of 3.0% non-fat dry milk. However, no pinking

agents were added in that experiment so it was impossible to discern whether the non-fat dry milk reduced pinking or the defect never formed. The a* value increased ($P<0.05$) with added PC. BP and ZA samples had lower ($P<0.05$) b* values compared to the control (9.14) while AP, CA, DA, EP, NM, PC, PY, and PZ had higher ($P<0.05$) b* values. An increase in the b* value was expected with NM because it was an inherently yellow powder and it was added in much greater quantity (3.0%) than the other ligands. It was unknown as to why several of the ligands increased the b* value.

Native Pigments and Hemochromes

The color of cooked meats may be related to, at least in part, the undenatured native pigments myoglobin, hemoglobin, and cytochrome c (Trout, 1989). Undenatured pigments can cause strong red colors when bound to the certain ligands (O_2, CO, NO). Ghorpade and Cornforth (1993) found that pork roasts cooked to 65°C exhibited a pink color which was determined (by reflectance spectroscopy) to be caused by undenatured myoglobin. Girard et al. (1990) reported that a pink color in turkey breast and pork loin cooked anywhere from 65° to 95°C was caused by cytochrome c.

Relative quantities of native pigments and hemochromes as calculated by manipulation of reflectance readings at specific wavelengths are presented in Table 2. Compared to the control, the level of DMb was decreased ($P<0.05$) in samples containing AP, CA, DA, EA, EP, NM, and PZ. BP was the only ligand sample that had an increased DMb. MbO_2 was decreased compared to the control ($P<0.05$) in samples containing AP, BP, CA, DA, EA, NM, PZ, and ZA. The MMb value was decreased (more metmyoglobin) in EP, PC, and PY samples and increased in CA, DA, EA, NM, and PZ

samples (less metmyoglobin). Fresh meat DMb, if present in sufficient quantity, gives the muscle a purple/red color. MbO_2 is responsible for the bright cherry red color of fresh red meats. MMb is the oxidized form of myoglobin and gives meat a brown appearance. When denatured, the fresh meat pigments are not able to produce the typical fresh meat colors. In the case of DMb and MbO_2, denaturation is desirable because a purple or red pigment would contribute to pinking in turkey.

Hemochromes are formed when a ligand binds to the heme iron of denatured muscle pigments. The only hemochrome measured in this section was NICHEM because there were no exogenous pinking agents added and nicotinamide is present in turkey at a higher level than other meats. NICHEM was lowered ($P<0.05$) in AP, CA, DA, and EA samples and increased ($P<0.05$) in BP samples (Table 2). Although BP resulted in the highest NICHEM (1.097), a similar result was not observed in the CIE a* values. However, PC had the highest numeric CIE a* value with a somewhat elevated NICHEM. A small increase in CIE a* value may result in a noticeably pinker sample while a larger increase in NICHEM may be needed to produce the same perceived increase in pinkness. CIE a* value may be more sensitive to increases in pinkness than NICHEM. The increase in NICHEM for BP may have been due to a synergistic affect of BP on nicotinamide binding. The reflectance spectrum of the BP-hemochrome may have also been similar to the nicotinamide hemochrome spectrum such that the NICHEM ratio also measured BP-hemochrome. A decrease in NICHEM may be due to the ligands binding the heme iron so that nicotinamide can not bind.

4.4.2 Effect of Ligands With Sodium Nitrite Added as a Pinking Agent

The second stage tested the effectiveness of the ligands at reducing a pink color caused by the addition of 150 ppm sodium nitrite. Low level sodium nitrite contamination has been demonstrated to cause pinking. The level of nitrite used here would never be present in an uncured product but it served to test the ability of the ligands to overcome an intense pink.

CIE L*a*b*

Many of the ligand samples had different ($P<0.05$) CIE L*a*b* values than the nitrite reference (Table 3). L* values were lower (control 73.70) for BP and PY and higher for CA, DA, EA, NM and ZA samples. The a* values were decreased (7.73 nitrite reference) for AP, DA, EA, EP, NM, PZ, and ZA samples. Higher a* values were seen for BP, PC, and PY. The b* values were lower (7.73 nitrite reference) in CA, DA, and EA samples and higher in AP, EP, and PZ treatments.

Native Pigments and Hemochromes

The native muscle pigments showed very little change in comparison to the control. ZA was the only sample with a lower DMb value. MbO_2 was decreased for AP, EP, NM, and PZ. The MMb value was lower for ZA (more metmyoglobin). Whatever the reason, ligands in the presence of nitrite did not have a profound effect on DMb, MbO_2, or MMb.

The value for Cure measured the relative amount of nitrosylhemochrome. This value was increased (less nitrosylhemochrome) for DA and NM samples. PC samples had

lower Cure values. None of the ligands had a significant effect on the NICHEM value (P>0.05). This may be due to nitric oxide binding the heme ring preferentially which did not allow native nicotinamide to bind.

4.4.3 Effect of Ligands With Nicotinamide Added as a Pinking Agent

The third stage tested the effectiveness of the ligands at reducing a pink color caused by the addition of 1.0% added nicotinamide. High levels of nicotinamide in turkey meat may be largely responsible for the pink defect. Added nicotinamide has been shown to produce a pink color (Cornforth et al, 1986; Claus et al., 1994) which makes it an ideal additive to test the pink color reduction ability of the ligands.

CIE L*a*b*

Many of the ligand samples had different (P<0.05) CIE L*a*b* values than the nicotinamide reference (Table 4). The BP and PY samples had lower L* values (73.24 nicotinamide reference) while the CA, DA, EA, and NM had higher L* values. The a* value (7.97 nicotinamide reference) was decreased for BP, CA, DA, EA, NM, PA, PZ, PY, YA, and ZA. The b* value (6.23 nicotinamide reference) was lower for BP and higher for CA, DA, EP, NM, PZ, and YA.

The percentage reduction in redness (CIE a*) by CA, DA, EA, and NM compared to the nicotinamide reference sample was 58.7%, 68.4%, 47.4%, and 72.4%, respectively. The next highest percentage reduction in a* was YA at 18.3%. The CIE a* values for these ligands (CA, DA, EA, NM) plus nicotinamide were reduced to a level below that of the control samples (4.21).

Native Pigments and Hemochromes

The DMb value was decreased in the BP, CA, DA, EA, EP, NM, PZ, and YA samples. MbO_2 was lowered in the BP, CA, DA, EA, NM, and PZ samples. MMb values were decreased (more metmyoglobin) in the CA, DA, EA, and NM samples.

The NICHEM value was 1.14 for the nicotinamide only samples. CA, DA, EA, NM, PZ, YA, and ZA significantly lowered ($P<0.05$) the NICHEM values.

4.3.4 Overall Effectiveness of Ligands

When evaluated over all three treatment groups (no pinking agent, sodium nitrite, nicotinamide) the most effective ligands at reducing pinking were CA, DA, EA, and NM. Samples with these four ligands were, in most cases, shown to have lower a* values, lower DMb, lower MbO_2, higher MMb, lower Cure, and lower NICHEM (Tables 2, 3, 4). These ligands had the most notable effect in reducing the a* value in the presence of 1.0% nicotinamide. The nicotinamide control had an a* value of 7.97 while the CA, DA, EA, and NM plus nicotinamide samples had a* values of 3.29, 2.52, 4.19, and 2.20 respectively. Numerically, these values are all lower than the straight control (a*, 4.21) although this statistical comparison was not made.

4.5 Conclusions

This experiment determined that it was possible to reduce the severity of pinking by the addition of non-pinking ligands to ground turkey. This pinking reduction by the ligands was seen when added alone and in the presence of sodium nitrite and especially

nicotinamide. CA, DA, EA, and NM were the four most effective ligands tested. The pink color reduction in nitrite samples was not apparent visually but there were modest reductions in the a* and Cure values. Ahn and Maurer (1987) found the amount of nitrite in raw turkey breast to be between 3.8 and 21 ppm. Sound GMP's should be able to reduce or eliminate external contamination of nitrite such that nitroso-pigments would not be a significant contributor to pinking. Because nicotinamide is endogenous to turkey at high levels, the ability of added ligands to reduce pinking due to nicotinamide hemochrome is of great importance. In general, pink color reduction was highest in the ligand only and the ligand plus nicotinamide samples as was observed by CIE a* and NICHEM value reductions.

4.6 References

Ahn, D.U. and Maurer, A.J. 1987. Concentration of nitrate and nitrite in raw turkey breast meat and the microbial conversion of added nitrate to nitrite in tumbled turkey breast meat. Poultry Sci. 66:1957-1959.

Ahn, D.U. and Maurer, A.J. 1990. Poultry meat color: kinds of heme pigments and concentrations of the ligands. Poultry Sci. 69:157-165.

Akoyunoglou, J-H.A., Olcott, H.S., and Brown, W.D. 1963. Ferrihemochrome and ferrohemochrome formation with amino acids, amino acid esters, pyridine derivatives and related compounds. Biochem. 2:1033-1041.

Brown, W. D. and Tappel, A.L. 1957. Identification of the pink pigment of canned tuna. Food Research. 22:214-219.

Claus, J.R., Shaw, D.E., and Marcy, J.A. 1994. Pink color development in turkey meat as affected by nicotinamide, cooking temperature, chilling rate, and storage time. J. Food Sci. 59:1283-1285.

Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., and Bartholomew, D.T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51:1132-1135.

Dobson, B.N. and Cornforth, D.P. 1992. Nonfat dry milk inhibits pink discoloration in turkey rolls. Poultry Sci. 71:1943-1946.

Froning, G.W. and Hartung, T.E. 1967. Effect of dietary nitrates and nitrites on color of chicken meat. Poultry Sci. 46:1261.

Froning, G.W., Daddario, J., Hartung, T.E., Sullivan, T.Wand Hill, R.M. 1969a. Color of poultry meat as influenced by dietary nitrates and nitrites. Poultry Sci. 48:668-674.

Froning, G.W., Mather, F.B., Daddario, J., and Hartung, T.E. 1969b. Effect of automobile exhaust fume inhalation by poultry immediately prior to slaughter on color of meat. Poultry Sci. 48:485-487.

Ghorpade, V.M. and Cornforth, D.P. 1993. Spectra of pigments responsible for pink color in pork roasts cooked to 65° or 82°C. J. Food Sci. 58:51-52, 89.

Girard, B., Vanderstoep, J., and Richards, J.F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55:1249-1254.

Mugler, D.J., Mitchell, J.D., and Adams, A.W. 1970. Factors affecting turkey meat color. Poultry Sci. 48:1510-1513.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. 31:68,69,72-44.

Richardson, M., Posati, L.P., and Anderson, B.A. 1980. Composition of foods. Sausage and luncheon meats (Raw, Processed, Prepared). Handbook No. 8-7. United States Department of Agriculture, Science and Education Administration. Washington D.C.

SAS, 1989. Statistical Analysis System Institute Inc., Cary, N.C.

Shaw, D.E., Claus, J.R., and Stewart, K.K. 1992. Effects of ammonia exposure on fresh pork: a distinct pink color after cooking. J. Muscle Foods 3:169-174.

Trout, G.R. 1989. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. J. Food Sci. 54:536-540.

Table 2- Ligand effects on instrumental color and pigment measurements in the absence of added pinking agents

Treatment[3]	CIE Values			Reflectance Values[1,2]			
	L*	a*	b*	DMb	MbO_2	MMb	NICHEM
Control	75.29^{cde}	4.21^{bc}	9.14^{d}	1.00^{bcd}	11.05^{ab}	1.33^{c}	1.000^{cd}
AP	74.97^{def}	3.73^{cd}	10.13^{ab}	0.97^{e}	9.77^{cd}	1.34^{bc}	0.993^{e}
BP	73.48^{f}	4.14^{bcd}	7.37^{f}	1.04^{a}	7.58^{fg}	1.32^{cd}	1.097^{a}
CA	77.01^{ab}	1.56^{e}	9.96^{abc}	0.96^{ef}	6.83^{g}	1.21^{f}	0.980^{e}
DA	77.19^{a}	1.95^{e}	10.22^{a}	0.96^{ef}	7.44^{g}	1.23^{ef}	0.980^{e}
DB	74.30^{def}	3.78^{cd}	9.39^{bcd}	0.98^{de}	10.04^{bcd}	1.31^{cd}	1.000^{cd}
EA	76.84^{abc}	2.25^{e}	9.26^{cd}	0.97^{e}	8.49^{ef}	1.25^{e}	0.987^{de}
EP	74.96^{def}	4.71^{b}	10.28^{a}	0.97^{e}	10.95^{ab}	1.37^{ab}	1.013^{bc}
NM	75.45^{bcd}	2.06^{e}	10.13^{ab}	0.94^{f}	7.73^{fg}	1.25^{e}	0.987^{de}
PA	75.43^{cd}	3.92^{cd}	9.03^{d}	1.00^{bcd}	10.44^{abc}	1.32^{cd}	1.003^{cd}
PC	73.96^{def}	5.58^{a}	10.03^{ab}	1.00^{bcd}	11.30^{a}	1.40^{a}	1.027^{cd}
PY	73.81^{ef}	4.82^{ab}	9.91^{abc}	1.00^{bc}	10.60^{abc}	1.38^{a}	1.003^{cd}
PZ	75.40^{cd}	3.42^{d}	10.57^{a}	0.96^{ef}	9.09^{de}	1.32^{cd}	0.990^{de}
YA	74.33^{def}	3.65^{cd}	9.39^{bcd}	0.98^{cde}	10.41^{abc}	1.32^{cd}	1.000^{cd}
ZA	76.72^{abc}	3.68^{cd}	8.17^{e}	1.00^{b}	9.78^{cd}	1.29^{d}	1.000^{cd}
Standard error	0.539	0.267	0.257	0.007	0.353	0.012	0.007

[a-k] means within the same column with different superscripts are different ($P<0.05$)

[1] Reflectance values - deoxymyoglobin (DMb) %R474/%R525, oxymyoglobin (MbO_2) %R630-%R580, metmyoglobin (MMb) %R630/%R650, nicotinamide hemochrome (nichem) %R537/%R553

[2] DMb, larger # = more; MbO_2, larger # = more; MMB, smaller # = more; NICHEM, larger # = more

[3] Treatment ligands- usage levels were 200 ppm except NM (NFDM, 3.0%)

Table 3- Ligand effects on instrumental color and pigment measurements in the presence of added sodium nitrite (150 ppm)

	CIE Values			Reflectance Values[1,2]				
Treatment[3]	L*	a*	b*	DMb	MbO_2	MMb	Cure	NICHEM
Sodium nitrite	73.70^{b}	7.17^{b}	7.73^{cd}	0.93^{a}	22.56^{abc}	1.49^{ab}	0.610^{cdef}	1.040^{a}
AP	72.88^{bc}	5.66^{f}	9.62^{a}	0.90^{ab}	18.88^{g}	1.45^{ab}	0.630^{bcd}	1.013^{a}
BP	72.30^{c}	8.39^{a}	7.45^{cdef}	0.94^{a}	23.32^{a}	1.57^{a}	0.570^{fg}	1.023^{a}
CA	76.19^{a}	6.89^{bcd}	6.65^{f}	0.95^{a}	22.97^{ab}	1.42^{b}	0.637^{bcd}	1.013^{a}
DA	75.90^{a}	6.59^{cde}	6.86^{ef}	0.95^{a}	22.49^{abc}	1.42^{b}	0.727^{a}	1.013^{a}
DB	72.89^{bc}	7.09^{bc}	7.91^{cd}	0.92^{a}	23.03^{ab}	1.48^{ab}	0.610^{cdef}	1.017^{a}
EA	75.25^{a}	6.21^{ef}	6.72^{f}	0.94^{a}	21.55^{cde}	1.41^{b}	0.647^{bc}	1.013^{a}
EP	73.54^{bc}	6.61^{cde}	9.01^{a}	0.91^{a}	20.57^{ef}	1.50^{ab}	0.603^{defg}	1.013^{a}
NM	75.11^{a}	5.67^{f}	8.22^{bc}	0.92^{a}	20.25^{f}	1.40^{b}	0.660^{b}	1.010^{a}
PA	73.42^{bc}	7.19^{b}	7.71^{cd}	0.92^{a}	23.46^{a}	1.48^{ab}	0.607^{cdefg}	1.013^{a}
PC	72.36^{bc}	8.37^{a}	7.46^{cdef}	0.93^{a}	23.51^{a}	1.56^{a}	0.567^{g}	1.017^{a}
PY	72.31^{c}	8.34^{a}	8.09^{bc}	0.93^{a}	23.32^{a}	1.56^{a}	0.573^{efg}	1.017^{a}
PZ	73.48^{bc}	6.53^{de}	8.80^{ab}	0.91^{a}	20.97^{def}	1.50^{ab}	0.607^{cdefg}	1.010^{a}
YA	73.60^{bc}	6.91^{bcd}	7.69^{cde}	0.92^{a}	23.39^{a}	1.48^{ab}	0.613^{cde}	1.013^{a}
ZA	75.28^{a}	5.81^{f}	7.12^{def}	0.82^{b}	21.90^{bcd}	1.24^{c}	0.647^{bc}	1.013^{a}
Standard error	0.477	0.190	0.288	0.031	0.417	0.046	0.014	0.006

[a-g] means within the same column with different superscripts are different ($P<0.05$)

[1] Reflectance values - deoxymyoglobin (DMb) %R474/%R525, oxymyoglobin (MbO_2) %R630-%R580, metmyoglobin (MMb) %R630/%R650, nitrosylhemochrome (cure) %R570/%R650, nicotinamide hemochrome (nichem) %R537/%R553

[2] DMb, larger # = more; MbO_2, larger # = more; MMB, smaller # = more; Cure, smaller # = more; NICHEM, larger # = more

[3] Treatment ligands- usage levels were 200 ppm except NM (NFDM, 3.0%)

Table 4- Treatment effects on instrumental color and pigment measurements in the presence of added nicotinamide (1.0%)

	CIE Values			Reflectance Values[1,2]			
Treatment[3]	L*	a*	b*	DMb	MbO_2	MMb	NICHEM
Nicotinamide	73.24^{ef}	7.97^{ab}	6.23^{gh}	1.08^{abcd}	11.59^{a}	1.47^{ab}	1.137^{abc}
AP	73.45^{de}	7.21^{bc}	7.43^{defg}	1.04^{e}	11.28^{ab}	1.46^{ab}	1.100^{cd}
BP	71.20^{g}	6.88^{c}	4.93^{I}	1.10^{a}	9.03^{cd}	1.42^{b}	1.163^{a}
CA	76.29^{ab}	3.29^{ef}	8.78^{bc}	0.98^{gh}	8.49^{de}	1.26^{c}	0.987^{f}
DA	76.52^{a}	2.52^{fg}	9.23^{ab}	0.97^{h}	8.44^{de}	1.25^{c}	0.987^{f}
DB	73.40^{e}	7.42^{bc}	6.87^{efgh}	1.05^{cde}	11.58^{a}	1.44^{ab}	1.113^{bcd}
EA	75.06^{abcd}	4.19^{e}	7.16^{degfh}	1.01^{fg}	9.78^{c}	1.17^{c}	1.040^{e}
EP	74.23^{cde}	7.20^{bc}	8.07^{bcde}	1.01^{ef}	11.63^{a}	1.47^{ab}	1.097^{cd}
NM	75.68^{abc}	2.20^{g}	10.12^{a}	0.93^{i}	7.73^{e}	1.26^{c}	0.987^{f}
PA	73.95^{de}	6.75^{c}	7.13^{degfh}	1.05^{de}	11.36^{ab}	1.41^{b}	1.103^{cd}
PC	71.73^{fg}	8.58^{a}	6.76^{fgh}	1.09^{ab}	11.79^{a}	1.53^{a}	1.153^{ab}
PY	71.43^{g}	8.57^{a}	6.69^{fgh}	1.19^{abc}	11.61^{a}	1.52^{a}	1.140^{abc}
PZ	74.42^{cde}	6.57^{c}	8.13^{bcd}	1.03^{ef}	10.68^{bc}	1.42^{b}	1.073^{de}
YA	74.25^{cde}	5.47^{d}	7.79^{cdef}	1.02^{ef}	11.26^{ab}	1.38^{b}	1.070^{de}
ZA	74.78^{bcde}	6.45^{c}	5.98^{hi}	1.06^{bcde}	11.25^{ab}	1.38^{b}	1.083^{de}
Standard error	0.573	0.337	0.431	0.012	0.273	0.035	0.016

[a-h] means within the same column with different superscripts are different ($P<0.05$)

[1] Reflectance values - deoxymyoglobin (DMb) %R474/%R525, oxymyoglobin (MbO_2) %R630-%R580, metmyoglobin (MMb) %R630/%R650, nicotinamide hemochrome (nichem) %R537/%R553

[2] DMb, larger # = more; MbO_2, larger # = more; MMB, smaller # = more; NICHEM, larger # = more

[3] Treatment ligands- usage levels were 200 ppm except NM (NFDM, 3.0%)

Chapter 5

Inhibition of Pinking in Cooked, Uncured Turkey Rolls Through the Binding of Non-Pinking Ligands to Muscle Pigments

5.1 Abstract

Consumers often assume that a pink color in uncured turkey is a result of undercooking. EDTA, CDTA, DTPA, and NFDM were tested in intact turkey breasts for their ability to reduce pinking with and without added pinking agents (nicotinamide or sodium nitrite). Ligands were evaluated at two or three different levels, over three storage times, and after two minutes of exposure light and air. In most cases, 50 ppm added ligand was sufficient to reduce pinking and higher levels were not beneficial. Ligands were more effective at reducing the pink color generated by nicotinamide than sodium nitrite. Overall, ligands delayed the onset pinking associated with storage time. In general, DTPA was the most effective at reducing pinking over all of the conditions of testing.

5.2 Introduction

There are many characteristics that consumers evaluate when making the decision to purchase poultry meats. Perhaps the most utilized characteristic is color because it is the easiest quality trait to evaluate. A major problem that has plagued the poultry industry, and other meat industries to a lesser degree, is a pink color defect. The pinking defect, also known as pink spot and pink ring, manifests itself as a pink coloring of the

fresh cut surface of meat products (Cornforth et al., 1986) such as cooked, uncured turkey and pork rolls. The pink pigment fades rapidly upon exposure to air and its occurrence is unpredictable. Because the pink color makes products appear undercooked, consumer complaints and commercial buyer discounting have been associated with products portraying the defect.

A wide variety of factors have been suggested as causes of pinking. Pool (1956) found that carbon monoxide and nitric oxide generated by the flames of gas ovens can combine with myoglobin to form a pink color. Likewise, exhaust fumes from automobiles (carbon monoxide and nitric oxide) inhaled by poultry enroute to processing facilities have been reported to cause a pink color (Froning et al., 1969b). Nitrates and nitrites in the feed (Froning and Hartung, 1967) or drinking water (Froning et al., 1969a) of poultry may lead to a pink end product. Nitrogenous contamination also can originate in the water utilized for carcass chilling (Mugler et al., 1970) and processing equipment previously used for cured products. Shaw et al. (1992) found that ammonia contamination caused a pink defect in pork.

It has been demonstrated that hemochromes can form by the interaction of nitrogenous ligands other than nitrates and nitrites. Certain amino acids (Akoyunoglou et al. 1963; Ahn and Maurer, 1990a), pyridine, and nicotinamide (Ahn and Maurer, 1990a) are just a few of the possible ligands that may form hemochromes with meat pigments. Akoyunoglou et al. (1963) characterized hemochromes as having a typical dual absorption spectrum with maxima at 520-530 nm and 555-565 nm. Cornforth et al. (1986) found that cooked turkey with 2.0% added nicotinamide had a reflectance spectrum similar to a

commercial pink turkey sample and it also exhibited the typical hemochrome absorption spectrum. Brown and Tappel (1957) observed a pink color in canned tuna and determined that the primary pigments were nicotinamide and denatured globin hemochromes. The high nicotinamide content of turkey meat (0.083 mg/g; Richardson et al., 1980) compared to other species favors the formation of nicotinamide hemochromes. Denatured proteins and free amino acids generated during the cooking process may also contribute to pinking through hemochrome formation.

Regardless of which native pigments or hemochromes are involved in the pinking defect, control of this problem is needed. Since pink generating hemochromes are formed when specific ligands bind the heme pigments, it may be possible to preferentially bind a ligand to the heme ring that does not cause a pink color. Schwarz et al. (unpublished data, 1996) tested fourteen compounds for their ability to reduce pinking in ground turkey with and without pinking agents (150 ppm sodium nitrite or 1.0% nicotinamide). It was found that trans 1,2-diaminocyclohexane-N,N,N',N' tetraacetic acid monohydrate (CDTA, 200 ppm); diethylenetriamine pentaacetic acid (DTPA, 200 ppm), ethylenedinitrilo-tetraacetic acid disodium salt (EDTA, 200 ppm), and calcium reduced non-fat dry milk (NFDM, 3.0%) were the most effective at reducing pinking. The objective of this research was to evaluate the pinking reduction ability of these four ligands (CDTA, DTPA, EDTA, NFDM) in intact turkey *Pectoralis major* muscles with and without sodium nitrite (0, 20,100 ppm) or nicotinamide (0, 1.0%) as pinking agents. Ligand efficacy was determined at various ligand levels and storage times. In addition, effect of ligands was

evaluated in relation to their impact on fading of the pink color upon exposure to light and air.

5.3 Materials and Methods

5.3.1 Raw material selection and characterization

Three day old unfrozen, boneless, skinless, turkey *Pectoralis major* muscles were acquired from a Virginia processor. Breasts for four replications were collected on four different production days. Breasts were visually selected to represent those with normal (non-dark) color. Each breast was measured for pH using the probe attachment of a pH meter (model Accumet 10, Fisher Scientific, Pittsburgh, Pennsylvania). Raw breasts had an average pH of 5.86 (standard deviation, 0.091). In addition, each breast was evaluated for CIE L*a*b* values with a chroma meter (model CR-200, Minolta Corp., Osaka, Japan) and a UV-visible-scanning spectrophotometer (model 2101PC, Shimadzu Inc., Kyoto, Japan). Each device was calibrated using a standard Minolta white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44; part # 20933026, Minolta Corp., Osaka, Japan). Three readings for each breast were taken using a 5 cm aperture held at a right angle to the breasts. Readings were taken from the ventral and dorsal sides of the breasts. The average CIE L*a*b* values were as follows: L* 52.84 (standard deviation, 2.743); a* 14.94 (standard deviation, 1.543); b* 3.20 (standard deviation, 1.111). After evaluation, each breast was stored separately in a moisture impermeable bag (type B540, Cryovac Division W.R. Grace & Co., Duncan, SC) overnight at 2°C.

5.3.2 Preparation of treatment solutions

Treatment solutions (ligands and other non-meat ingredients) were prepared for injection (20% meat weight basis, MWB) into the breasts. All solutions had added sodium chloride (NaCl) and sodium tripolyphosphate (STP) so that the 20% injection provided 2.0% NaCl and 0.5% STP (MWB). The single-component solutions and control were as follows (all values MWB): control (no additional additives), sodium nitrite (20 ppm), sodium nitrite (100 ppm), nicotinamide (1.0%), ligand (CDTA, DTPA, EDTA; at 50 ppm, 100 ppm, and 200 ppm), and NFDM (1.0%, and 2.0%). The remaining solutions were all of the combinations of the ligands (all three levels) or NFDM (both levels) with sodium nitrite (both levels) or nicotinamide.

5.3.3 Preparation of turkey rolls

All visible external fat was removed from the breasts and two lean strips (totaling approximately 240 g) cut parallel to the long axis were removed. Next, the breasts were injected 20% with their respective treatment solutions using a 60.0 ml syringe with a 16 gage needle. Injections were made both parallel and perpendicular to the muscle strips. Once injected, the breasts were placed in moisture impermeable bags (type B450, Cryovac Division W.R. Grace & Co., Duncan, SC) and tumbled for one h (MC80-226, Inject Star, Globus Co., Austria) before being refrigerated overnight. Each injected breast was tumbled again for 1 h, weighed, and stuffed into 3.18 cm diameter, 30.48 cm long stainless steel tubes (2.6 mm thick) for cooking. The submerged ends of the tubes were sealed with rubber stoppers and the other ends were covered with aluminum foil. The tubes were then

placed in a 90°C water bath (custom built, Virginia Tech) for cooking to an end-point temperature of 80°C. Temperature of the meat was monitored by three, 15.24 cm spike thermocouples inserted into placebo meat stuffed tubes and attached to a data logger (Model 5100, Electronic Controls Design, Milwaukee, Oregon). After cooking, the breasts were chilled rapidly to 7°C in an ice bath and refrigerated (2°C) overnight.

5.3.4 Evaluation of cooked breasts

After chilling, each cooked breast sample was removed from its tube and weighed to determine a combined cooking and chilling loss. Next, each breast sample was sliced into three equal pieces and separately vacuum packaged at setting 6 (model VC999/01, Inauen Maschinen AG, Herisau, Switzerland) in moisture impermeable bags (type B540, Cryovac Division W.R. Grace & Co., Duncan, SC). The chunks were stored in the dark at 2°C for 1, 14, and 21 days. After storage, the chunks were evaluated for pH and color. Each sample (5 g portions) was homogenized in distilled water (1 g:10 ml) using a Virtishear with a macro-fine generator attached to a 20 mm shaft (item number 225318, Virtis Co., Inc., Gardiner, NY) and measured for pH (Accumet pH meter 10, Fisher Scientific Co., Pittsburgh, Pennsylvania).

Color evaluation consisted of CIE L*a*b* measurements (model CR-200, Minolta Corp., Osaka, Japan), reflectance spectroscopy in the visible, 400-700 nm, range (model 2101PC, Shimadzu Inc., Kyoto, Japan), and sensory panel evaluation. The 1 cm aperture of the chroma meter was held at right angle to sample. The spectrophotometer was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm, and fast scan speed.

Each device was calibrated using a standard Minolta white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44; part # 20933026, Minolta Corp., Osaka, Japan). The CIE L*a*b* and reflectance readings were taken on the fresh cut and light faded surface (1076 Lux fluorescent light for 2 min) of each sample. Light intensity was measured with a light meter (model L096080, Extech Instrument Corp., Waltham, Massachusetts). The sensory panel (8 judges) was trained (3 sessions) to recognize different degrees of pinkness. Training consisted of evaluation of untreated, sodium nitrite treated, and nicotinamide treated samples. Four photographic reference pictures (CIE a* values of 1.59, 5.43, 12.70, 17.70) from not pink to extremely pink was utilized as a reference during the training and evaluation sessions. The reference pictures were placed near a 38.1 cm (15 inch line representing the 15 cm line used during judging) so that increasing degrees of pinkness were represented. Sensory evaluation was performed on day 14 samples (reps 2, 3, and 4) under fluorescent lighting (1076 Lux). Because there were 48 samples, each repetition's evaluation was split into two sessions (24 samples/session). Each sample was presented as a 1 cm disc which was placed on a black paper backdrop adjacent to the reference pictures. The outer edge (1-2 mm) of each sample was covered by placing a piece of black paper with the center cut out. Scores for each sample were recorded by placing a mark on a 15 cm line with vertical marks at each end and at 7.5 cm. The line anchors were the terms "not pink" and "extremely pink". Each sample was removed after 30 sec because, in some cases, the pink color faded rapidly.

5.3.5 Statistical analysis

Data from the dependent variables (CIE L*a*b*, NICHEM, Cure) for CDTA, DTPA, and EDTA were analyzed by the GLM procedure (SAS, 1989) using a 2x4 factorial (nicotinamide level and ligand level) or a 3x4 factorial (sodium nitrite level and ligand level) split-split plot design. When the ligand was NFDM, a 2x3 or 3x3 factorial (nicotinamide or sodium nitrite level and NFDM level) split-split plot design was used. For the overall effectiveness of CDTA, DTPA, and EDTA, a 2x3 or 3x3 factorial (nicotinamide or sodium nitrite level and ligand type) spilt-split plot was used. To compare all four ligands, the ligand levels, days stored, and time readings were all averaged so that a 2x3 or 3x3 factorial (nicotinamide or sodium nitrite level and ligand type) design could be used. The sensory panel data for CDTA, DTPA, and EDTA were analyzed using a 2x4 factorial (nicotinamide level and ligand level) or a 3x4 factorial (sodium nitrite level and ligand level) block design. When the ligand was NFDM, a 2x3 or 3x3 factorial (nicotinamide or sodium nitrite level and NFDM level) plot design was used. For all designs, the F statistic and P values for the effects of the ligand treatments were generated. When significance ($P<0.05$) was determined in the model, means were separated by the Least Significant Difference procedure of SAS (1989).

5.4 Results and Discussion

5.4.1 Effect of each ligand on sample pH and native pigments

The pH of raw turkey may vary from bird to bird. In addition, processing conditions (slaughter or product manufacture) can lead to changes in the pH that affect

ligand binding to the heme pigments. Ahn and Maurer (1990b) found that the addition of 0.1M phosphate to a pigment solution increased the pH by 0.4 units and in turn increased heme complex forming reactions. An increase in pH raises the end-point temperature necessary to denature muscle pigments. If this pH affect is not accounted for, undenatured pigments will be available to produce a red color in the cooked product. Phosphate (0.5% on meat weight basis) was added to the turkey rolls in this project and a pH increase of approximately 0.45 units (5.85 to 6.30) was observed. Since all samples had the same level of added phosphate, the pH increase caused by the phosphate was relatively uniform across all samples. The pH of the each sample was taken after cooking to determine if the addition of the various ligands had an effect. Overall, the addition of ligands did not change the pH of the samples with and without pinking agents (nicotinamide or sodium nitrite) present. The nicotinamide set had pH's of 6.30, 6.26, 6.31, and 6.32 for CDTA, DTPA, EDTA, and NFDM, respectively. The nitrite set had pH's of 6.32, 6.28, 6.30, and 6.33 for CDTA, DTPA, EDTA, and NFDM, respectively. If a ligand had significantly raised the pH of the samples, the formation of a pink hemochrome would be favored and would warrant disqualification of the ligand.

5.4.2 Effect of each ligand on looking loss

Cooking loss is an economically important characteristic that the industry monitors because loss of moisture translates to loss of product weight and, consequently, reduced profits. In addition, water soluble compounds (pigments, added ligands, salt, etc.) may be lost along with water. In this project, the ligands that were added to reduce pinking could

have been lost in the purge thus it was important that the ligands themselves did not increase water loss. There were no differences (P>0.05) in cooking loss for any of the ligands in samples with the ligands alone, added nicotinamide plus ligands, or added sodium nitrite plus ligands. The level of the ligand added had no effect on the cooking loss. The average cooking loss for all samples was 13.6% (standard deviation , 2.8). Any differences in pinkness of the samples was not due to ligand loss through purge.

5.4.3 Effect of ligand level of each ligand on dependent variables

Determination of the minimum quantity of ligand necessary to significantly reduce pinking was desirable. This may be important relative to nutritional concerns of adding non-meat ingredients that may make certain minerals less available. In addition, excess ligand without added benefit would be economically inefficient.

Without Pinking Agents

CIE a*: Incorporation of CDTA and DTPA resulted in lower (P<0.05) CIE a* values than the control with an average reduction of 29.1% and 31.7%, respectively (Table 1). CIE a* values tended (P>0.05) to decrease as the ligand level increased from 50 to 200 ppm for CDTA and DTPA. Incorporation of EDTA did not decrease (P>0.05) CIE a* values compared to the control. Samples containing 2.0% NFDM had lower (P<0.05) CIE a* values than the control while those samples containing 1.0% NFDM were not different.

NICHEM: Samples containing 50 to 200 ppm CDTA or EDTA had lower (P<0.05) NICHEM compared to the control (Table 1). A minimum of 100 ppm DTPA

was required to produce a NICHEM less (P<0.05) than the control. NFDM containing samples were not different (P>0.05) than the control. With the exception of NFDM, NICHEM tended (P<0.05) to decrease as the ligand level increased from 50 to 200 ppm.

Sensory: Samples containing 50 to 200 ppm CDTA produced lower sensory (P<0.05) scores than the control (Table 4). Increasing the level of CDTA did not change (P>0.05) the sensory pinkness scores. At least 100 ppm DTPA was necessary to lower (P<0.05) the sensory score below the control. None of the EDTA or NFDM levels changed (P>0.05) the sensory scores in comparison to the control.

CIE L* and b*: CDTA containing samples (50 to 200 ppm) had higher (P<0.05) CIE L* values than the control (Table 1). Greater than 100 ppm DTPA was required to increase (P<0.05) the CIE L* value while EDTA and NFDM did not change (P>0.05) the CIE L* value in comparison to the control. In general, the addition of ligands resulted in either higher or similar CIE b* values to the control (Table 1). Higher CIE b* values may be due to non-pinking ligands shifting the wavelengths of light absorbed and thus, the color perceived.

With Nicotinamide as a Pinking Agent

Because the pinking defect is a sporadically occurring problem, it was necessary to add a pinking agent to some of the samples to ensure the development of a pink color so that the impact of added ligands could be determined. A major pigment identified in the pinking defect of turkey is the nicotinamide hemochrome (Cornforth et al., 1986). Nicotinamide at 2.0% will cause an intense pink color in cooked turkey (Claus et al.,

1994; Cornforth et al., 1986). A lower level of 1.0% was found to cause an intense pink color in turkey (Schwarz et al., 1996 unpublished data) and was used in this project.

CIE a*: Levels of DTPA from 50 to 200 ppm resulted in lower ($P<0.05$) CIE a* values than the nicotinamide reference (Table 2) with an average reduction of 30.8%. CDTA was effective at lowering the CIE a* value at 50 and 200 ppm but not at 100 ppm. Incorporation of EDTA at greater than 50 ppm lowered ($P<0.05$) the CIE a* values while at least 2.0% NFDM was required for the same result. The effectiveness of the ligand should be strongly tied to its affinity for the heme iron. CDTA and DTPA have been used specifically in medical therapy to bind iron. However, DTPA may have a greater affinity because it is a pentaacetic acid whereas CDTA is a tetraacetic acid. EDTA is a general cationic chelator and may be less effective because of its lower affinity for iron.

NICHEM: DTPA treated samples had lower ($P<0.05$) NICHEM than the nicotinamide reference from 50 to 200 ppm (Table 2). At least 2.0% NFDM was needed to lower NICHEM. Neither CDTA nor EDTA were different ($P>0.05$) from the nicotinamide reference. Ligands had different affects on the CIE a* and NICHEM values. CIE a* measures all pigments (fresh pigments, nicotinamide hemochrome, denatured protein hemochromes, nitrosylhemochrome, etc.) contributing to the red color of meat. Therefore it was possible for a ligand, for example, to reduce the NICHEM value without affecting the CIE a* value.

Sensory: DTPA at 100 ppm was the only ligand level to lower ($P<0.05$) the sensory pinkness score in relation to the nicotinamide reference (Table 4). CDTA, EDTA,

and NFDM were not different (P>0.05) than the reference, although the numeric means for the maximum ligand level tended to be lower.

CIE L* and b*: CDTA and DTPA, at all levels, increased (P<0.05) the CIE L* values (Table 2) in comparison to the nicotinamide reference. EDTA, at 200 ppm, resulted in the only different and higher (P<0.05) CIE L* than the reference. NFDM samples were not different (P>0.05) from the reference. Generally, the addition of ligands resulted in either higher or similar CIE b* values compared to the reference (Table 2).

With Sodium Nitrite as a Pinking Agent

Ahn and Maurer (1987) found the amount of nitrite in raw turkey breast to be between 3.8 and 21 ppm. This nitrite may have originated from the diet and/or drinking water of the live birds (Froning et al., 1969a), carcass chill water (Mugler et al., 1970), or from manufacturing equipment which had been previously used for cured products. The chances of significant nitrite contamination should be limited by good manufacturing practices but it is necessary to know the effectiveness of the ligands at reducing the pink color should contamination occur.

CIE a*: Although sodium nitrite was added at 20 and 100 ppm, there were no differences in pinkness between these two levels. Therefore, only means averaged across nitrite level will be discussed. No differences (P>0.05) in CIE a* values were observed for any of the ligands at any level of use in comparison to nitrite references.

Cure: Incorporation of 200 ppm DTPA lowered (P<0.05) the Cure (nitrosylhemochrome) compared to the nitrite reference (Table 3). CDTA, EDTA and

NFDM samples were not different (P>0.05) than the reference. Nitric oxide is a much smaller ligand with a greater affinity for heme iron than many other pinking agents or ligands. These factors may make it more competitive than the ligands for the iron binding site and reduce the efficacy of the ligands in pink reduction.

NICHEM: NFDM samples at 1.0% and 2.0% had lower (P<0.05) NICHEM than the nitrite reference (Table 3). Samples with CDTA, DTPA, and EDTA were not different (P>0.05) in NICHEM than the nitrite reference.

Sensory: Incorporation of EDTA from 50 to 200 ppm lowered (P<0.05) the sensory pinkness scores (Table 4). CDTA (100 ppm) and DTPA (50 ppm) also had lower (P<0.05) sensory scores but higher levels of these ligands were not different from the nitrite reference.

CIE L* and b*: The CIE L* values were not different (P>0.05) from the nitrite reference for any of the ligands (Table 3). Addition of EDTA (200 ppm) produced a lower CIE b* value while NFDM (1.0% and 2.0%) produced samples with higher (P<0.05) CIE b* values than the nitrite reference.

5.4.4 Effect of storage time on dependent variables for each ligand

It has been shown that a freshly cooked product that exhibits no pink may develop the defect with refrigerated storage (Claus et al., 1994). This is thought to be associated with the development of reducing conditions in the meat as storage time increases. In general, commercial turkey and pork rolls are stored for several days (usually weeks)

which may be sufficient for pinking to develop. The severity of the storage time generated pink may be lowered by the addition of one or more of these ligands.

Without Pinking Agents

CIE a*: Incorporation of EDTA and NFDM (all levels) produced samples that did not differ (P>0.05) in CIE a* value as storage time increased from 1 to 21 days (Table 5). When CDTA was added to samples, CIE a* values were not different (P>0.05) up to 14 days. DTPA samples had similar (P>0.05) CIE a* values for 1 and 14 days but day 21 had a higher (P<0.05) a* value. Cornforth (1989) reported that in the center of large roasts or in vacuum packaged products, where oxygen is absent, the denatured metmyoglobin may be gradually reduced (ie. the heme iron is converted to the ferrous form) with refrigerated storage. After 14 to 21 days, the affinity of iron for pinking agents (e.g. nicotinamide) in the CDTA and DTPA samples may have been greater than the affinity for the non-pinking ligands which resulted in an increase in pinking. The ligands competed well with pinking agents through 21 days in EDTA and NFDM samples.

NICHEM: There were few differences in NICHEM over storage time for any of the ligands (Table 5). Day 21 samples, in the presence of CDTA, were higher (P<0.05) than the day 1 samples. Again, there were many components measured in the CIE a* value (not just nicotinamide hemochrome) and it was possible for the a* value to change without a change in NICHEM.

CIE L* and b*: NFDM treated samples had higher (P<0.05) CIE L* values for day 14 and 21 (Table 5). There were no differences in CIE b* value for any of the ligands

as storage time increased from day 1 to 21 (Table 5). Even though the CIE L*a*b* measurements are independent, in general, as CIE a* values increased (decreased), the CIE L* and b* values decreased (increased).

With Nicotinamide as a Pinking Agent

CIE a*: The CIE a* values did not change (P>0.05) with storage time in NFDM samples (Table 6). NFDM may have inhibited pink generation by competitive binding to the heme ring or by masking an increase in pink because of the high amount added (2.0%). CIE a* values for 1 and 14 days in DTPA samples were the same (P>0.05) but they increased (P<0.05) at 21 days. This observation indicated that DTPA may have delayed the onset of pinking. EDTA samples had higher (P<0.05) CIE a* values at day 14 and 21. Samples treated with CDTA showed a steady increase (P<0.05) in CIE a* values from 1 to 21 days. NFDM and DTPA may have remained bound to the iron longer than EDTA and CDTA which made them more effective at delaying pinking. After 21 days, though, pinking agents may have out-competed ligands for the heme iron.

NICHEM: EDTA containing samples had similar (P>0.05) NICHEM values through day 14 (Table 6). By delaying the formation of NICHEM, EDTA produced a short term reduction in pinking. CDTA and NFDM treated samples had higher (P<0.05) NICHEM at days 14 and 21. NICHEM increased (P<0.05) with storage time in the presence of DTPA. The results were opposite from the CIE a* value results. This means that some ligands were efficient at reducing nicotinamide hemochrome but the CIE a*

value still increased. The CIE a* increase may be due to the formation of denatured protein hemochromes or nitrosylhemochrome from nitrite contamination.

CIE L* and b*: CIE L* values did not change (P>0.05) for any of the ligands as storage time increased (Table 6). In general, CIE b* values decreased as storage time increased. The increase in CIE b* values may be the result of non-pinking ligands competitively replacing the nicotinamide which shifts what wavelengths of light are absorbed or reflected. Also, if oxygenated, undenatured myoglobin was initially present, this pigment would be converted to deoxymyoglobin with storage time.

With Sodium Nitrite as a Pinking Agent

CIE a*: When samples were treated with EDTA and NFDM, no differences (P>0.05) in CIE a* values were observed as storage time increased (Table 7). Samples with added CDTA and DTPA were not different (P>0.05) from day 1 to 14 in CIE a* values. This indicated that CDTA and DTPA had a delaying effect on pinking. The nitrite treated samples were very pink immediately after cooking. Therefore, as storage time increased, the increase in pink was low because most of the color had already been generated.

Cure: Treatment with NFDM produced samples with more (P<0.05) nitrosylhemochrome (lower Cure value) at day 14 and 21 (Table 7). CDTA, DTPA, and EDTA treated samples did not differ (P>0.05) as storage time increased. As with the CIE a* value, most of the nitrosylhemochrome pigment was formed early on such that only a limited amount could be formed with storage time.

NICHEM: NICHEM increased (P<0.05) from 1 to 14 days in DTPA and NFDM treated samples (Table 7). CDTA and EDTA samples did not differ (P>0.05)in NICHEM as storage time increased. The increase in NICHEM up to day 14 may have been due to early competitiveness of nicotinamide. As the meat reduced with time, nitrite may have out-competed nicotinamide.

CIE L* and b*: Generally, CIE L* and b* values decreased or remained the same as storage time increased from 1 to 21 days (Table 7). As the samples became more red, they darkened and yellow generating ligands could not bind as much.

5.4.5 Effect of each ligand on fading time

The pink color generated in cooked, uncured turkey rolls has been found to be unstable. Shortly after a product is cut the pink color may fade as a result of exposure to air and light (Howe et al., 1982, Cornforth et al., 1986). This fading may be caused by the same autoxidation and photo-oxidation that causes cured products to fade (Walsh and Rose, 1957). The effect of each ligand on the degree of pink fading was measured at 2 min after the samples were sliced. Sliced samples were held under 1076 Lux fluorescent lighting for the 2 min. It was hypothesized that the ligands may increase fading of the pink color. This would benefit to the industry because the pink color could be essentially gone before the consumers perceived the problem.

Without Pinking Agents

The CIE a* values decreased for each ligand (Table 8). Control products faded approximately 1.0 CIE a* unit while ligand treated samples faded approximately 0.35 units. It should be noted that the starting level of pinkness in ligand treated samples was generally lower than the controls. Therefore, these samples did not have as much pink color to fade. The ligand treated samples always had lower ($P<0.05$) faded CIE a* values than the controls. While the degree of pink fading was less for ligand treated samples, the overall pinkness was less when ligands were added.

As the samples faded, the quantity of nicotinamide hemochrome (NICHEM) decreased ($P<0.05$) for all ligands (Table 8). The CIE L* values generally remained unchanged. The CIE b* values increased for all ligands.

With Nicotinamide as a Pinking Agent

When nicotinamide was added as a pinking agent, results similar to the ligand only samples were observed (Table 9). The CIE a* and NICHEM values decreased as the samples faded. In general, the CIE L* and CIE b* values increased after the samples were faded.

With Sodium Nitrite as a Pinking Agent

When sodium nitrite was added to the samples, 2 min was generally not enough time to produce significant fading. In general, there was no change in the CIE L*a*b*, Cure, or NICHEM after 2 min values for any of the ligands (Table 10). With respect to

degree of pinking, NFDM faded samples had a lower ($P<0.05$) CIE a* value and CDTA and EDTA treated samples had lower ($P<0.05$) NICHEM values. These results indicated that fading in nitrite treated samples did not contribute significantly to the reduction of pinking with any of the ligands except NFDM.

5.4.6 Effectiveness of ligands over all levels, storage times, and fading times

After all individual ligand effects were determined, it was important to determine which ligand was the most effective overall at reducing pinking. When tested alone (without pinking agents), all of the ligands were similar in their pink color reducing ability. (Table 11). The sensory scores for CDTA were lower ($P<0.05$) than the other ligands.

When nicotinamide was added to the samples, DTPA was one of the most effective at reducing pinking. The CIE a*, NICHEM, and sensory values were consistently low for DTPA (Table 11). The effectiveness of the ligand should be strongly tied to its affinity for the heme iron. CDTA and DTPA have been used specifically in medical therapy to bind iron. However, DTPA may have a greater affinity because it is a pentaacetic acid whereas CDTA is a tetraacetic acid.

All of the ligands had similar effects on the pink color dependent variables (CIE a*, Cure, NICHEM) in the nitrite treated samples (Table 11). NFDM produced the highest CIE b* values in nitrite containing samples. This increase in yellow may have been due to the inherent yellow color of the NFDM or denatured NFDM may be a yellow forming ligand.

5.5 Conclusion

Reduction in pinking was characterized by a reduction in the dependent variables: CIE a*, NICHEM, nitrosylhemochrome, or sensory scores. Individual ligands did not affect all of the dependent variables in the same manner. In addition, some ligands exhibited more pink color reduction without pinking agents while others were more effective with pinking agents. In general, ligands were more effective at reducing pinking generated by nicotinamide than pinking caused by sodium nitrite. Nitrite may bind the heme ring more preferentially than the ligands because of its smaller size and greater affinity for the heme ring. Ligands may have been more competitive with nicotinamide and therefore reduced pinking more effectively in its presence.

In general, low levels of added ligand (50 ppm) produced a majority of the pink color reduction such that higher levels (100, 200 ppm) were not significantly more beneficial. Some ligands delayed the pinking associated with increasing storage time. This effect was demonstrated best in samples without added pinking agents but nicotinamide samples were affected similarly. Without pinking agents, the ligands dramatically reduced sensory pinkness scores.

One of the best ligands at reducing pinking under a majority of conditions tested was DTPA. Because the ligands affected the dependent variables differently, it may be beneficial to utilize several at once to produce cooperative pinking reduction.

5.6 References

Ahn, D.U. and Maurer, A.J. 1987. Concentration of nitrate and nitrite in raw turkey breast meat and the microbial conversion of added nitrate to nitrite in tumbled turkey breast meat. Poultry Sci. 66:1957-1959.

Ahn, D.U. and Maurer, A.J. 1990a. Poultry meat color: kinds of heme pigments and concentrations of the ligands. Poultry Sci. 69:157-165.

Ahn, D.U. and Maurer, A.J. 1990b. Poultry meat color: pH and the heme-complex forming reaction. Poultry Sci. 69:2040-2050.

Akoyunoglou, J-H.A., Olcott, H.S., and Brown, W.D. 1963. Ferrihemochrome and ferrohemochrome formation with amino acids, amino acid esters, pyridine derivatives and related compounds. Biochem. 2:1033-1041.

Brown, W. Duane and Tappel, A.L. 1957. Identification of the pink pigment of canned tuna. Food Research. 22:214-219.

Claus, J.R., Shaw, D.E., and Marcy, J.A. 1994. Pink color development in turkey meat as affected by nicotinamide, cooking temperature, chilling rate, and storage time. J. Food Sci. 59:1283-1285.

Cornforth, D.P. 1989. Color-fresh, cooked, cured. Western States Meat Association Annual Meat Processing Conference. 41-53.

Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., and Bartholomew, D.T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51:1132-1135.

Froning, G.W. and Hartung, T.E. 1967. Effect of dietary nitrates and nitrites on color of chicken meat. Poultry Sci. 46:1261.

Froning, G.W., Daddario, J., Hartung, T.E., Sullivan, T.Wand Hill, R.M. 1969a. Color of poultry meat as influenced by dietary nitrates and nitrites. Poultry Sci. 48:668-674.

Froning, G.W., Mather, F.B., Daddario, J., and Hartung, T.E. 1969b. Effect of automobile exhaust fume inhalation by poultry immediately prior to slaughter on color of meat. Poultry Sci. 48:485-487.

Howe, J.L., Gullett, E.A., Usborne, W.R. 1982. Development of pink color in cooked pork. Can. Inst. Food Sci. and Tech. 15:19-23.

Mugler, D.J., Mitchell, J.D., and Adams, A.W. 1970. Factors affecting turkey meat color. Poultry Sci. 48:1510-1513.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. 31:68,69,72-44.

Richardson, M., Posati, L.P., and Anderson, B.A. 1980. Composition of foods. Sausage and luncheon meats (Raw, Processed, Prepared). Handbook No. 8-7. United States Department of Agriculture, Science and Education Administration. Washington D.C.

SAS, 1989. Statistical Analysis System Institute Inc., Cary, NC.

Shaw, D.E., Claus, J.R., and Stewart, K.K. 1992. Effects of ammonia exposure on fresh pork: a distinct pink color after cooking. J. Muscle Foods 3:169-174.

Walsh, K.A. and Rose, D. 1957. Factors affecting the oxidation of nitric oxide myoglobin. Agric. Food Chem. 4:352-355.

Table 1- Effect of ligand and ligand level[1] on color and nicotinamide hemochrome[2] for cooked turkey breasts without pinking agents[3]

Ligand	Ligand Level	CIE L*	CIE a*	CIE b*	NICHEM
CDTA	0 ppm	78.25^{b}	5.97^{a}	6.54^{c}	1.054^{a}
	50 ppm	79.46^{a}	4.51^{b}	7.08^{bc}	1.031^{b}
	100 ppm	79.87^{a}	4.18^{b}	7.79^{a}	1.026^{b}
	200 ppm	79.51^{a}	4.01^{b}	7.15^{b}	1.022^{b}
	S.E.	0.323	0.268	0.182	0.006
DTPA	0 ppm	78.25^{b}	5.97^{a}	6.54^{b}	1.054^{a}
	50 ppm	77.72^{b}	4.69^{b}	6.55^{b}	1.034^{ab}
	100 ppm	78.96^{ab}	4.10^{b}	7.67^{ab}	1.016^{bc}
	200 ppm	80.09^{a}	3.44^{b}	7.81^{a}	1.011^{c}
	S.E.	0.523	0.399	0.379	0.007
EDTA	0 ppm	78.25^{a}	5.97^{a}	6.54^{c}	1.054^{a}
	50 ppm	79.85^{a}	4.57^{a}	6.80^{bc}	1.031^{b}
	100 ppm	79.02^{a}	4.66^{a}	7.49^{ab}	1.026^{b}
	200 ppm	77.18^{a}	5.01^{a}	7.77^{a}	1.023^{b}
	S.E.	1.064	0.437	0.295	0.007
NFDM	0%	78.25^{a}	5.97^{a}	6.54^{b}	1.054^{a}
	1%	77.11^{a}	5.41^{ab}	7.62^{ab}	1.030^{a}
	2%	77.68^{a}	4.56^{b}	8.64^{a}	1.033^{a}
	S.E.	0.523	0.403	0.415	0.007

$^{a-c}$ means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across days (1, 14, 21) and fading times (0, 2 min)

[1] ligands added at specified levels on a meat weight basis

[2] nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), higher value = more NICHEM

[3] pinking agents, nicotinamide and sodium nitrite, not added

Table 2- Effect of ligand and ligand level[1] on color and nicotinamide hemochrome[2] for cooked turkey breasts with nicotinamide as a pinking agent[3]

		CIE			
Ligand	**Ligand Level**	**L***	**a***	**b***	**NICHEM**
CDTA	0 ppm	74.93^{b}	11.52^{a}	2.79^{b}	1.234^{a}
	50 ppm	76.98^{a}	8.30^{b}	4.53^{ab}	1.168^{a}
	100 ppm	77.24^{a}	9.20^{ab}	4.32^{ab}	1.155^{a}
	200 ppm	77.54^{a}	8.11^{b}	5.13^{a}	1.166^{a}
	S.E.	0.517	0.750	0.582	0.029
DTPA	0 ppm	74.93^{b}	11.52^{a}	2.79^{b}	1.234^{a}
	50 ppm	76.92^{a}	8.85^{b}	4.45^{a}	1.145^{b}
	100 ppm	77.79^{a}	7.52^{b}	4.94^{a}	1.121^{b}
	200 ppm	77.56^{a}	7.53^{b}	5.27^{a}	1.126^{b}
	S.E.	0.596	0.723	0.425	0.024
EDTA	0 ppm	74.93^{b}	11.52^{a}	2.79^{b}	1.234^{a}
	50 ppm	76.42^{ab}	9.97^{ab}	3.34^{ab}	1.216^{a}
	100 ppm	76.63^{ab}	7.40^{c}	4.69^{ab}	1.153^{a}
	200 ppm	77.14^{a}	7.68^{bc}	5.27^{a}	1.149^{a}
	S.E.	0.536	0.749	0.604	0.028
NFDM	0%	74.93^{a}	11.52^{a}	2.79^{b}	1.234^{a}
	1%	76.94^{a}	10.02^{ab}	4.25^{ab}	1.201^{ab}
	2%	75.57^{a}	8.31^{b}	5.36^{a}	1.170^{b}
	S.E.	0.886	0.801	0.523	0.018

[a-c] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across days (1, 14, 21) and fading times (0, 2 min)

[1] ligands added at specified levels on a meat weight basis

[2] nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), higher value = more NICHEM

[3] nicotinamide added at 1.0% on a meat weight basis

Table 3- Effect of ligand and ligand level[1] on color and hemochrome[2] values for cooked turkey breasts with sodium nitrite[3]

Ligand	Ligand Level	CIE L*	CIE a*	CIE b*	Cure	NICHEM
CDTA	0 ppm	76.96^{a}	7.25^{a}	5.30^{a}	0.630^{ab}	1.020^{a}
	50 ppm	77.46^{a}	7.36^{a}	5.44^{a}	0.627^{ab}	1.020^{a}
	100 ppm	77.95^{a}	6.98^{a}	5.18^{a}	0.646^{a}	1.019^{a}
	200 ppm	77.86^{a}	7.12^{a}	5.53^{a}	0.623^{b}	1.019^{a}
	S.E.	0.441	0.286	0.136	0.007	0.001
DTPA	0 ppm	76.96^{a}	7.25^{a}	5.30^{a}	0.630^{b}	1.020^{a}
	50 ppm	77.05^{a}	6.60^{a}	5.17^{a}	0.646^{ab}	1.019^{a}
	100 ppm	77.81^{a}	7.04^{a}	5.25^{a}	0.630^{b}	1.018^{a}
	200 ppm	78.28^{a}	6.77^{a}	5.37^{a}	0.648^{a}	1.017^{a}
	S.E.	0.697	0.243	0.230	0.005	0.001
EDTA	0 ppm	76.96^{a}	7.25^{a}	5.30^{a}	0.630^{a}	1.020^{a}
	50 ppm	77.57^{a}	7.26^{a}	5.35^{a}	0.633^{a}	1.019^{a}
	100 ppm	77.42^{a}	7.08^{a}	5.58^{a}	0.636^{a}	1.019^{a}
	200 ppm	77.87^{a}	6.68^{a}	4.86^{b}	0.640^{a}	1.020^{a}
	S.E.	0.634	0.290	0.124	0.007	0.001
NFDM	0%	76.96^{a}	7.25^{a}	5.30^{c}	0.630^{a}	1.020^{a}
	1%	77.22^{a}	6.66^{a}	6.07^{b}	0.649^{a}	1.015^{b}
	2%	76.28^{a}	6.67^{a}	7.05^{a}	0.640^{a}	1.012^{b}
	S.E.	0.318	0.240	0.107	0.008	0.001

[a-c] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across days (1, 14, 21) and fading times (0, 2 min)

[1] ligands added at specified levels on a meat weight basis

[2] hemochromes are nitrosylhemochrome (Cure, %R570 nm/%R650 nm) and nicotinamide hemochrome (NICHEM, %R537nm /%R553 nm), lower Cure and higher NICHEM values = more pigment, respectively

[3] mean value for sodium nitrite 20 and 100 ppm samples used because no color difference was found between these levels

Table 4- Mean sensory scores[1] for each ligand and ligand level[2] with and without a pinking agent[3]

Ligand	Ligand Level	Sensory Score: No pinking agent	Sensory Score: Nicotinamide 1%	Sensory Score: Sodium Nitrite[4]
CDTA	0	4.53[a]	11.00[a]	5.77[a]
	50	0.37[b]	10.77[a]	5.18[ab]
	100	0.43[b]	11.33[a]	3.83[b]
	200	0.33[b]	7.47[a]	4.82[ab]
	S.E.	0.436	2.165	0.518
DTPA	0	4.53[a]	11.00[a]	5.77[a]
	50	2.20[ab]	9.03[ab]	2.75[b]
	100	1.63[b]	5.00[b]	4.08[ab]
	200	0.40[b]	7.90[ab]	4.07[ab]
	S.E.	0.685	1.187	0.578
EDTA	0	4.53[a]	11.00[a]	5.77[a]
	50	1.67[a]	10.50[a]	3.68[b]
	100	1.43[a]	11.13[a]	4.27[b]
	200	3.07[a]	9.63[a]	3.67[b]
	S.E.	1.061	1.324	0.344
NFDM	0	4.53[a]	11.00[a]	5.77[a]
	1%	1.97[a]	8.03[a]	3.87[a]
	2%	1.93[a]	7.40[a]	4.55[a]
	S.E.	1.060	1.456	0.733

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$), sodium nitrite means for 20 and 100 ppm columns were analyzed as one set

[1] scores are centimeter measurements on a 15 cm unstructured line from not pink to extremely pink

[2] ligands added on a meat weight basis (MWB)

[3] pinking agents are nicotinamide (added at 1.0% MWB and sodium nitrite (mean score from 20 ppm and 100 ppm samples)

Table 5- Effect of ligand and storage time[1] on color and nicotinamide hemochrome[2] for cooked turkey breasts without a pinking agent[3]

Ligand	Storage (days)	CIE			NICHEM
		L*	a*	b*	
CDTA	1	79.15[a]	4.40[b]	7.29[a]	1.029[ab]
	14	79.41[a]	4.56[ab]	7.07[a]	1.026[b]
	21	79.25[a]	5.04[a]	7.06[a]	1.044[a]
	S.E.	0.211	0.169	0.182	0.006
DTPA	1	78.94[a]	4.17[b]	7.30[a]	1.021[a]
	14	78.56[a]	4.49[b]	7.12[a]	1.031[a]
	21	78.76[a]	4.98[a]	7.01[a]	1.033[a]
	S.E.	0.220	0.110	0.166	0.005
EDTA	1	78.88[a]	4.79[a]	7.39[a]	1.028[a]
	14	78.86[a]	5.21[a]	7.04[a]	1.038[a]
	21	77.97[a]	5.15[a]	7.01[a]	1.033[a]
	S.E.	0.605	0.201	0.173	0.006
NFDM	1	76.97[b]	4.99[a]	7.75[a]	1.035[a]
	14	78.17[a]	5.31[a]	7.53[a]	1.046[a]
	21	77.90[a]	5.63[a]	7.53[a]	1.036[a]
	S.E.	0.217	0.277	0.200	0.007

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 or 1.0%, 2.0%) and fading times (0, 2 min)

[1] samples stored under vacuum at 2°C in moisture impermeable bags for 1, 14, and 21 days

[2] nicotinamide hemochrome (NICHEM, R537 nm/%R553 nm), higher value = more NICHEM

[3] pinking agents, nicotinamide and sodium nitrite, not added

Table 6- Effect of ligand and storage time[1] on color and nicotinamide hemochrome[2] for cooked turkey breasts with nicotinamide as a pinking agent[3]

		CIE			
Ligand	**Storage (days)**	**L***	**a***	**b***	**NICHEM**
CDTA	1	76.60^{a}	7.33^{c}	5.50^{a}	1.111^{b}
	14	76.34^{a}	9.47^{b}	3.91^{b}	1.196^{a}
	21	77.08^{a}	11.04^{a}	3.17^{b}	1.234^{a}
	S.E.	0.354	0.422	0.330	0.016
DTPA	1	77.13^{a}	7.42^{b}	5.35^{a}	1.088^{c}
	14	76.67^{a}	8.67^{b}	4.31^{b}	1.162^{b}
	21	76.60^{a}	10.48^{a}	3.43^{c}	1.219^{a}
	S.E.	0.222	0.440	0.288	0.017
EDTA	1	76.54^{a}	7.60^{b}	5.12^{a}	1.139^{b}
	14	75.84^{a}	9.61^{a}	3.80^{b}	1.177^{b}
	21	76.46^{a}	10.23^{a}	3.15^{b}	1.248^{a}
	S.E.	0.268	0.386	0.369	0.020
NFDM	1	75.87^{a}	9.42^{a}	4.26^{a}	1.142^{b}
	14	75.68^{a}	9.91^{a}	4.34^{a}	1.233^{a}
	21	75.88^{a}	10.52^{a}	3.80^{a}	1.230^{a}
	S.E.	0.328	0.490	0.369	0.019

[a-c] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 or 1.0% 2.0%) and fading times (0, 2 min)

[1] samples stored under vacuum at 2°C in moisture impermeable bags for 1, 14, and 21 days

[2] nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), higher value = more NICHEM

[3] nicotinamide added at 1.0% on a meat weight basis

Table 7- Effect of ligand and storage time[1] on color and hemochrome[2] values for cooked turkey breasts with sodium nitrite[3]

Ligand	Storage (days)	CIE L*	CIE a*	CIE b*	Cure	NICHEM
CDTA	1	77.96^{a}	6.93^{b}	5.55^{a}	0.640^{a}	1.019^{a}
	14	77.42^{ab}	7.18^{ab}	5.17^{b}	0.626^{a}	1.020^{a}
	21	77.29^{b}	7.42^{a}	5.36^{ab}	0.628^{a}	1.020^{a}
	S.E.	0.206	0.109	0.103	0.005	0.001
DTPA	1	78.01^{a}	6.78^{b}	5.43^{a}	0.642^{a}	1.017^{b}
	14	76.96^{a}	6.80^{b}	5.03^{b}	0.637^{a}	1.019^{a}
	21	77.60^{a}	7.17^{a}	5.36^{a}	0.636^{a}	1.020^{a}
	S.E.	0.491	0.109	0.100	0.005	0.001
EDTA	1	77.89^{a}	7.00^{a}	5.42^{a}	0.636^{a}	1.018^{a}
	14	76.90^{b}	7.02^{a}	5.08^{b}	0.638^{a}	1.020^{a}
	21	77.58^{ab}	7.19^{a}	5.31^{ab}	0.631^{a}	1.020^{a}
	S.E.	0.308	0.123	0.090	0.005	0.001
NFDM	1	76.92^{a}	6.75^{a}	6.03^{a}	0.652^{a}	1.014^{b}
	14	76.80^{a}	6.72^{a}	6.22^{a}	0.632^{b}	1.017^{a}
	21	76.74^{a}	7.10^{a}	6.18^{a}	0.634^{b}	1.017^{a}
	S.E.	0.266	0.153	0.101	0.005	0.001

$^{a\text{-}b}$ means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 or 1.0% 2.0%) and fading times (0, 2 min)

[1] samples stored under vacuum at 2°C in moisture impermeable bags for 1, 14, and 21 days

[2] hemochromes are nitrosylhemochrome (Cure, %R570 nm/%R650 nm) and nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), lower Cure and higher NICHEM values = more pigment, respectively

[3] mean value for sodium nitrite 20 ppm and 100 ppm treated samples

Table 8- Effect of ligand and fading time[1] on color and nicotinamide hemochrome[2] for cooked turkey breasts without a pinking agent[3]

Ligand	Fading time (min)	CIE L*	CIE a*	CIE b*	NICHEM
CDTA	0	79.17^b	4.97^a	6.86^b	1.043^a
	2	79.37^a	4.37^b	7.42^a	1.023^b
	S.E.	0.050	0.045	0.040	0.002
DTPA	0	78.74^a	4.79^a	6.87^b	1.038^a
	2	78.77^a	4.30^b	7.41^a	1.019^b
	S.E.	0.043	0.037	0.043	0.002
EDTA	0	78.15^a	5.39^a	6.84^b	1.043^a
	2	79.00^a	4.71^b	7.46^a	1.023^b
	S.E.	0.528	0.044	0.065	0.002
NFDM	0	77.68^a	5.61^a	7.32^b	1.050^a
	2	77.68^a	5.02^b	7.88^a	1.027^b
	S.E.	0.036	0.043	0.042	0.003

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100,200 ppm or 1.0%, 2.0%) and storage time (1, 14, 21 days)

[1] samples analyzed immediately after cutting and after 2 minutes of exposure to 1076 Lux of fluorescent light

[2] nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), high value = more NICHEM

[3] pinking agents, nicotinamide and sodium nitrite, not added

Table 9- Effect of ligand and fading time[1] on color and nicotinamide hemochrome[2] for cooked turkey breasts with nicotinamide as a pinking agent[3]

Ligand	Fading time (min)	CIE L*	CIE a*	CIE b*	NICHEM
CDTA	0	76.46[b]	10.03[a]	3.58[b]	1.212[a]
	2	76.88[a]	8.54[b]	4.81[a]	1.149[b]
	S.E.	0.049	0.086	0.059	0.006
DTPA	0	76.73[a]	9.53[a]	3.81[b]	1.180[a]
	2	76.87[a]	8.17[b]	4.91[a]	1.130[b]
	S.E.	0.087	0.066	0.065	0.004
EDTA	0	76.17[b]	9.90[a]	3.42[b]	1.219[a]
	2	76.39[a]	8.39[b]	4.63[a]	1.157[b]
	S.E.	0.039	0.061	0.064	0.005
NFDM	0	75.67[b]	10.82[a]	3.48[b]	1.243[a]
	2	75.95[a]	9.08[b]	4.79[a]	1.160[b]
	S.E.	0.051	0.079	0.065	0.006

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100,200 ppm or 1.0%, 2.0%) and storage time (1, 14, 21 days)

[1] samples analyzed immediately after cutting and after 2 minutes of exposure to 1076 Lux of fluorescent light

[2] nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), high value = more NICHEM

[3] nicotinamide added at 1.0% on a meat weight basis

Table 10- Effect of ligand and fading time[1] on color and hemochrome[2] values for cooked turkey breasts with sodium nitrite[3]

Ligand	Fading time (min)	CIE L*	CIE a*	CIE b*	Cure	NICHEM
CDTA	0	77.54[a]	7.26[a]	5.27[a]	0.630[a]	1.021[a]
	2	77.58[a]	7.09[a]	5.45[a]	0.633[a]	1.018[b]
	S.E.	0.137	0.104	0.078	0.003	0.001
DTPA	0	77.19[a]	6.98[a]	5.18[a]	0.638[a]	1.019[a]
	2	77.86[a]	6.85[a]	5.37[a]	0.639[a]	1.018[a]
	S.E.	0.390	0.082	0.076	0.002	0.001
EDTA	0	76.62[a]	7.14[a]	5.18[b]	0.635[a]	1.021[a]
	2	77.30[a]	7.00[a]	5.36[a]	0.635[a]	1.019[b]
	S.E.	0.298	0.086	0.056	0.002	0.001
NFDM	0	76.86[a]	6.92[a]	6.03[b]	0.641[a]	1.016[a]
	2	76.79[a]	6.79[b]	6.25[a]	0.639[a]	1.016[a]
	S.E.	0.045	0.019	0.018	0.002	0.001

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 ppm or 1.0%, 2.0%) and storage time (1, 14, 21 days)

[1] samples analyzed immediately after cutting and after 2 minutes of exposure to 1076 Lux of fluorescent light

[2] hemochromes are nitrosylhemochrome (Cure, %R570 nm/%R650 nm) and nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), lower Cure and higher NICHEM values = more pigment, respectively

[3] mean value for sodium nitrite 20 ppm and 100 ppm treated samples

Table 11- Overall[1] effect of each ligand on color and hemochrome[2] values for cooked turkey breasts with and without a pinking agent[3]

Category	CDTA	DTPA	EDTA	NFDM	S.E.
No pinking agent					
CIE					
L*	79.27^{a}	78.75^{a}	78.57^{a}	77.68^{a}	0.528
a*	4.67^{a}	4.55^{a}	5.05^{a}	5.31^{a}	0.291
b*	7.14^{a}	7.14^{a}	7.15^{a}	7.60^{a}	0.168
NICHEM	1.033^{a}	1.029^{a}	1.033^{a}	1.039^{a}	0.003
Sensory	1.42^{b}	2.19^{ab}	2.68^{a}	2.81^{a}	0.323
Nicotinamide 1.0%					
CIE					
L*	76.67^{a}	76.80^{a}	76.28^{ab}	75.81^{b}	0.238
a*	9.28^{ab}	8.85^{b}	9.14^{b}	9.95^{a}	0.229
b*	4.19^{a}	4.36^{a}	4.02^{a}	4.14^{a}	0.236
NICHEM	1.181^{ab}	1.156^{b}	1.188^{ab}	1.202^{a}	0.013
Sensory	10.14^{ab}	8.23^{c}	10.57^{a}	8.81^{bc}	0.430
Sodium nitrite[4]					
CIE					
L*	77.56^{a}	77.53^{ab}	77.46^{ab}	76.82^{b}	0.220
a*	7.18^{a}	6.91^{a}	7.07^{a}	6.86^{a}	0.180
b*	5.36^{b}	5.27^{b}	5.27^{b}	6.14^{a}	0.074
Cure	0.631^{a}	0.638^{a}	0.635^{a}	0.640^{a}	0.004
NICHEM	1.020^{a}	1.019^{a}	1.020^{a}	1.016^{b}	0.001
Sensory	4.900^{a}	4.167^{a}	4.346^{a}	4.723^{a}	0.333

[a-c] means for each ligand with different superscripts and within same row are different ($P<0.05$)

[1] Ligand levels (50, 100, 200 ppm for CDTA, DTPA, EDTA and 1.0%, 2.0% for NFDM), storage times (1, 14, 21 days), and fading time (1, 2 min) pooled for CIE L*a*b*, Cure, and NICHEM values

[2] hemochromes are nitrosylhemochrome (Cure, %R570 nm/%R650 nm) and nicotinamide hemochrome (NICHEM, %R537 nm/%R553 nm), lower Cure and higher NICHEM values = more pigment, respectively

[3] pinking agents are nicotinamide (1.0% on a meat weight basis) and sodium nitrite (20 and 100 ppm on a meat weight basis)

[4] mean value for sodium nitrite 20 ppm and 100 ppm treated samples

Chapter 6

Summary and Conclusions

The development of a simple method to quantify the presence of nicotinamide hemochrome was necessary in light of the potential role of this pigment in the pinking defect of cooked, uncured turkey. Reflectance spectroscopy has been one of the primary methods for identifying the presence of nicotinamide hemochromes and extending that methodology to quantify this pigment was logical. Through the correlation of reflectance wavelength ratios of nicotinamide treated turkey, the ratio of %R537 nm /%R553 nm was found to represent the relative level of nicotinamide hemochrome.

Pinking in ground turkey was reduced by adding non-pinking ligands. The ligands reduced pinking when added alone, with sodium nitrite and especially with nicotinamide. Because nicotinamide is endogenous to turkey at high levels, the ability of added ligands to reduce pinking due to nicotinamide hemochrome was of great importance. CDTA, DTPA, EDTA, and NFDM were the most effective ligands identified.

The four ligands (CDTA, DTPA, EDTA, NFDM) were evaluated for pink color reduction in intact turkey breasts. Individual ligands did not affect all of the dependent variables (CIE a*, NICHEM, nitrosylhemochrome, or sensory scores) in the same manner. In addition, some ligands exhibited more pink color reduction without pinking agents while others were more effective with pinking agents. In general, ligands were more effective at reducing pinking generated by nicotinamide than that caused by sodium nitrite. The ligands and nicotinamide had similar affinity for the heme ring which permitted a

reduction in pinking. However, nitrite may preferentially bind to the heme ring because it was a smaller that had a greater affinity for the heme ring than the ligands. In general, low levels of added ligand (50 ppm) produced a majority of the pink color reduction such that higher levels (100, 200 ppm) were not significantly more beneficial. Some ligands delayed the pinking associated with increasing storage time. This effect was demonstrated best in samples without added pinking agents but nicotinamide samples were affected similarly. Without pinking agents, the ligands dramatically reduced sensory pinkness scores.

One of the best ligands at reducing pinking under a majority of conditions tested was DTPA. Because the ligands affected the dependent variables differently, it may be beneficial to combine several of the ligands to produce cooperative pinking reduction.

Chapter 7

Appendix

7.1 Myoglobin Concentration

Myoglobin concentration was measured using a modification procedure of Trout (1989). The raw meat myoglobin level was not measured because undenatured myoglobin concentration, not percent myoglobin denatured, was desired. Centrifugation of the phosphate-meat slurry was performed at 48,000xg instead of 50,000xg.

The average myoglobin concentration of all cooked samples was 0.281 mg/g (standard deviation; 0.165). In general, there was no difference ($P>0.05$) in myoglobin concentration because of added ligand, ligand level, or pinking agent.

7.2 Effect of Each Ligand on Native Myoglobin Derivatives

Relative quantities of the dependent variables deoxymyoglobin (%R474 nm / %R 525 nm), oxymyoglobin (%R630 nm - %R580 nm), and metmyoglobin (%R630 nm / %R 525 nm) were calculated by manipulation of percent reflectance at specific wavelengths. Chemical state of these pigments was important because of their impact on pinking. Ferrous pigments are redder whereas ferric pigments are brown. If present in sufficient quantity, these pigments could contribute to the color of cooked meat.

The effects of ligand level and storage time on the level and chemical state of these pigments, with and without pinking agents, are illustrated in Tables 1-6. Tables 7-9 depict the effects of fading on these variables.

Table 1- Effect of ligand and ligand level[1] on myoglobin derivatives[2] for cooked turkey breasts without pinking agents[3]

		CIE		
Ligand	**Ligand Level**	**DMb**	**MbO_2**	**MMb**
CDTA	0 ppm	1.08^a	13.41^a	1.38^a
	50 ppm	1.06^b	12.29^b	1.33^b
	100 ppm	1.03^b	11.63^b	1.30^b
	200 ppm	1.04^b	11.99^b	1.31^b
	S.E.	0.007	0.311	0.011
DTPA	0 ppm	1.08^a	13.41^a	1.38^a
	50 ppm	1.06^{ab}	11.63^b	1.32^b
	100 ppm	1.03^b	11.43^b	1.30^b
	200 ppm	1.03^b	11.01^b	1.28^b
	S.E.	0.010	0.450	0.015
EDTA	0 ppm	1.08^a	13.41^a	1.38^a
	50 ppm	1.04^b	11.91^a	1.30^b
	100 ppm	1.05^b	12.31^a	1.34^{ab}
	200 ppm	1.04^b	12.14^a	1.33^{ab}
	S.E.	0.009	0.590	0.023
NFDM	0%	1.08^a	13.41^a	1.38^a
	1%	1.05^b	12.80^a	1.36^a
	2%	1.04^b	12.84^a	1.35^a
	S.E.	0.008	0.294	0.010

[a-c] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across days (1, 14, 21) and fading times (0, 2 min)

[1] ligands added at specified levels on a meat weight basis

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/%R525 nm

[3] pinking agents, nicotinamide and sodium nitrite, not added

Table 2- Effect of ligand and ligand level[1] on myoglobin derivatives[2] for cooked turkey breasts with nicotinamide as a pinking agent[3]

Ligand	Ligand Level	CIE		
		DMb	MbO_2	MMb
CDTA	0 ppm	1.19[a]	13.56[a]	1.53[a]
	50 ppm	1.13[a]	13.25[a]	1.47[a]
	100 ppm	1.14[a]	12.97[a]	1.47[a]
	200 ppm	1.14[a]	12.86[a]	1.46[a]
	S.E.	0.019	0.505	0.040
DTPA	0 ppm	1.19[a]	13.56[a]	1.53[a]
	50 ppm	1.12[ab]	12.90[a]	1.43[ab]
	100 ppm	1.11[b]	12.35[a]	1.40[b]
	200 ppm	1.11[b]	12.52[a]	1.41[ab]
	S.E.	0.023	0.434	0.039
EDTA	0 ppm	1.19[a]	13.56[a]	1.52[a]
	50 ppm	1.18[a]	12.98[a]	1.50[a]
	100 ppm	1.14[a]	12.77[a]	1.44[a]
	200 ppm	1.14[a]	12.96[a]	1.44[a]
	S.E.	0.022	0.416	0.038
NFDM	0%	1.08[a]	13.41[a]	1.38[a]
	1%	1.05[b]	12.80[a]	1.36[a]
	2%	1.04[b]	12.84[a]	1.35[a]
	S.E.	0.008	0.294	0.010

[a-c] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across days (1, 14, 21) and fading times (0, 2 min)

[1] ligands added at specified levels on a meat weight basis

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/%R525 nm

[3] nicotinamide added at 1.0%

Table 3- Effect of ligand and ligand level[1] on myoglobin derivatives[2] for cooked turkey breasts with sodium nitrite as a pinking agent[3]

		CIE		
Ligand	**Ligand Level**	**DMb**	**MbO_2**	**MMb**
CDTA	0 ppm	0.98^{a}	23.80^{ab}	1.43^{ab}
	50 ppm	0.97^{a}	24.36^{a}	1.43^{ab}
	100 ppm	0.98^{a}	23.12^{b}	1.40^{b}
	200 ppm	0.98^{a}	24.38^{a}	1.44^{a}
	S.E.	0.003	0.354	0.012
DTPA	0 ppm	0.98^{a}	23.80^{a}	1.43^{a}
	50 ppm	0.98^{a}	23.09^{a}	1.40^{b}
	100 ppm	0.98^{a}	23.97^{a}	1.43^{a}
	200 ppm	0.97^{a}	23.06^{a}	1.40^{b}
	S.E.	0.002	0.325	0.009
EDTA	0 ppm	0.98^{a}	23.80^{a}	1.43^{a}
	50 ppm	0.96^{a}	23.68^{a}	1.42^{a}
	100 ppm	0.97^{a}	23.80^{a}	1.41^{a}
	200 ppm	0.98^{a}	23.71^{a}	1.40^{a}
	S.E.	0.010	0.442	0.012
NFDM	0%	0.98^{a}	23.80^{a}	1.43^{a}
	1%	0.96^{b}	22.56^{a}	1.41^{a}
	2%	0.95^{c}	22.65^{a}	1.43^{a}
	S.E.	0.002	0.42	0.010

[a-c] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across days (1, 14, 21) and fading times (0, 2 min)

[1] ligands added at specified levels on a meat weight basis

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/%R525 nm

[3] sodium nitrite added at 20 and 100 ppm

Table 4- Effect of ligand and storage time[1] on myoglobin derivatives[2] for cooked turkey breasts without pinking agents[3]

Ligand	Storage (days)	DMb	MbO_2	MMb
CDTA	1	1.05[ab]	11.94[a]	1.32[a]
	14	1.04[b]	12.25[a]	1.32[a]
	21	1.07[a]	12.79[a]	1.35[a]
	S.E.	0.007	0.333	0.011
DTPA	1	1.04[a]	11.58[b]	1.31[a]
	14	1.05[a]	11.54[b]	1.31[a]
	21	1.06[a]	12.49[a]	1.33[a]
	S.E.			
EDTA	1	1.05[a]	11.98[a]	1.33[a]
	14	1.06[a]	12.66[a]	1.34[a]
	21	1.05[a]	12.68[a]	1.34[a]
	S.E.	0.006	0.264	0.008
NFDM	1	1.05[a]	12.47[b]	1.35[a]
	14	1.07[a]	13.22[ab]	1.37[a]
	21	1.06[a]	13.36[a]	1.37[a]
	S.E.	0.010	0.293	0.011

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 or 1.0%, 2.0%) and fading times (0, 2 min)

[1] samples stored under vacuum at 2°C in moisture impermeable bags for 1, 14, and 21 days

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/ %R525 nm

[3] pinking agents, nicotinamide and sodium nitrite, not added

Table 5- Effect of ligand and storage time[1] on myoglobin derivatives[2] for cooked turkey breasts with nicotinamide as a pinking agent[3]

Ligand	Storage (days)	DMb	MbO_2	MMb
CDTA	1	1.10^b	12.60^b	1.41^b
	14	1.16^a	13.24^{ab}	1.49^a
	21	1.19^a	13.64^a	1.54^a
	S.E.	0.014	0.278	0.023
DTPA	1	1.08^b	11.80^b	1.38^b
	14	1.14^a	13.21^a	1.45^a
	21	1.17^a	13.48^a	1.51^a
	S.E.	0.013	0.346	0.021
EDTA	1	1.13^b	12.55^b	1.42^b
	14	1.15^{ab}	13.24^{ab}	1.48^{ab}
	21	1.20^a	13.42^a	1.53^a
	S.E.	0.016	0.263	0.023
NFDM	1	1.17^b	12.45^b	1.44^b
	14	1.18^a	13.45^a	1.53^a
	21	1.17^{ab}	13.93^a	1.53^a
	S.E.	0.017	0.300	0.259

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 or 1.0%, 2.0%) and fading times (0, 2 min)

[1] samples stored under vacuum at 2°C in moisture impermeable bags for 1, 14, and 21 days

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/ %R525 nm

[3] nicotinamide added at 1.0%

Table 6- Effect of ligand and storage time[1] on myoglobin derivatives[2] for cooked turkey breasts with sodium nitrite as a pinking agent[3]

Ligand	Storage (days)	DMb	MbO_2	MMb
CDTA	1	0.98[a]	23.43[a]	1.41[a]
	14	0.97[a]	24.14[a]	1.43[a]
	21	0.98[a]	24.17[a]	1.43[a]
	S.E.	0.002	0.309	0.010
DTPA	1	1.04[a]	11.58[b]	1.31[a]
	14	1.05[a]	11.54[b]	1.31[a]
	21	1.06[a]	12.48[a]	1.33[a]
	S.E.	0.007	0.287	0.009
EDTA	1	0.98[a]	23.87[a]	1.41[a]
	14	0.96[a]	23.36[a]	1.41[a]
	21	0.97[a]	24.01[a]	1.42[a]
	S.E.	0.009	0.272	0.009
NFDM	1	0.96[a]	22.48[a]	1.41[a]
	14	0.96[a]	23.20[a]	1.43[a]
	21	0.96[a]	23.34[a]	1.43[a]
	S.E.	0.002	0.395	0.009

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100, 200 or 1.0%, 2.0%) and fading times (0, 2 min)

[1] samples stored under vacuum at 2°C in moisture impermeable bags for 1, 14, and 21 days

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/ %R525 nm

[3] sodium nitrite added at 20 and 100 ppm

Table 7- Effect of ligand and fading time[1] on myoglobin derivatives[2] for cooked turkey breasts without pinking agents[3]

Ligand	Fading time (min)	DMb	MbO_2	MMb
CDTA	0	1.07^a	12.48^a	1.34^a
	2	1.04^b	12.18^b	1.32^b
	S.E.	0.003	0.060	0.002
DTPA	0	1.06^a	11.90^a	1.32^a
	2	1.04^b	11.84^a	1.31^b
	S.E.	0.002	0.064	0.002
EDTA	0	1.06^a	12.56^a	1.34^a
	2	1.04^b	12.32^b	1.33^b
	S.E.	0.002	0.079	0.003
NFDM	0	1.07^a	13.12^a	1.37^a
	2	1.05^b	12.91^a	1.35^b
	S.E.	0.004	0.097	0.003

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100,200 ppm or 1.0%, 2.0%) and storage time (1, 14, 21 days)

[1] samples analyzed immediately after cutting and after 2 minutes of exposure to 1076 Lux of fluorescent light

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/ %R525 nm

[3] pinking agents, nicotinamide and sodium nitrite, not added

Table 8- Effect of ligand and fading time[1] on myoglobin derivatives[2] for cooked turkey breasts with nicotinamide as a pinking agent[3]

Ligand	Fading time (min)	DMb	MbO_2	MMb
CDTA	0	1.18[a]	13.01[b]	1.51[a]
	2	1.13[b]	13.31[a]	1.46[b]
	S.E.	0.005	0.082	0.005
DTPA	0	1.15[a]	12.68[b]	1.46[a]
	2	1.11[b]	12.99[a]	1.42[b]
	S.E.	0.004	0.064	0.005
EDTA	0	1.18[a]	12.89[b]	1.50[a]
	2	1.14[b]	13.25[a]	1.46[b]
	S.E.	0.004	0.062	0.005
NFDM	0	1.18[a]	13.17[b]	1.52[a]
	2	1.13[b]	13.38[a]	1.47[b]
	S.E.	0.004	0.064	0.005

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100,200 ppm or 1.0%, 2.0%) and storage time (1, 14, 21 days)

[1] samples analyzed immediately after cutting and after 2 minutes of exposure to 1076 Lux of fluorescent light

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/ %R525 nm

[3] nicotinamide added at 1.0%

Table 9- Effect of ligand and fading time[1] on myoglobin derivatives[2] for cooked turkey breasts with sodium nitrite as a pinking agent[3]

Ligand	Fading time (min)	DMb	MbO_2	MMb
CDTA	0	0.98[a]	22.99[a]	1.42[a]
	2	0.97[a]	23.83[a]	1.42[a]
	S.E.	0.002	0.183	0.005
DTPA	0	0.98[a]	23.53[a]	1.41[a]
	2	0.98[a]	23.43[a]	1.42[a]
	S.E.	0.001	0.129	0.004
EDTA	0	0.98[a]	23.78[a]	1.41[a]
	2	0.97[a]	23.71[a]	1.42[a]
	S.E.	0.007	0.150	0.004
NFDM	0	0.96[a]	23.01[a]	1.42[a]
	2	0.96[a]	23.00[a]	1.43[a]
	S.E.	0.001	0.088	0.002

[a-b] means for each ligand with different superscripts and within same column are different ($P<0.05$). Means were pooled across ligand levels (50, 100,200 ppm or 1.0%, 2.0%) and storage time (1, 14, 21 days)

[1] samples analyzed immediately after cutting and after 2 minutes of exposure to 1076 Lux of fluorescent light

[2] DMb = %R 474 nm/%R525 nm; MbO_2 = %R 630 nm-%R 580 nm; MMb = %R630 nm/ %R525 nm

[3] sodium nitrite added at 20 and 100 ppm

7.3 Reference

Trout, G.R. 1989. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. J. Food Sci. 54:536-540.

Vita

Stephen Joseph Schwarz was born on September 2, 1970 in Baltimore, Maryland, and is the son of Richard and Lisa Eager. He received his high school diploma from North Carroll High School in Hampstead, Maryland and his Bachelor of Science degree in Animal Science in May, 1993 from Virginia Polytechnic Institute and State University. After graduation, Stephen attended veterinary school for one year before transferring into the Food Science graduate program at Virginia Polytechnic Institute and State University. While in his masters program, Stephen and his wife Rita had their first child, Tobias Joseph. Stephen and his family moved to South Carolina where he began employment with Cryovac Division of W.R. Grace and Co.

Stephen J Schwarz