THE ROLE OF INGREDIENTS AND PROCESSING CONDITIONS ON MARINADE PENETRATION, RETENTION AND COLOR DEFECTS IN COOKED MARINATED CHICKEN BREAST MEAT

by

EDWIN YPARRAGUIRRE PALANG

(Under the Direction of Romeo T. Toledo)

ABSTRACT

Marination by vacuum tumbling is commonly practiced in the meat industry; however, the optimal temperature of the process to maximize marinade absorption and retention is yet to be defined. Furthermore, the role of marinade functional ingredients and pH of the marinade in alleviating problems with pink color in cooked meat and water retention during cooking is yet to be elucidated.

For denaturation model study, myoglobin pigments from chicken gizzards were extracted. Observed results from the model study were validated in whole muscle and comminuted chicken meat. In addition, temperature of vacuum tumbling operation was optimized with respect to marinade pick-up, cook yield and expressible moisture. Also, trivalent Eu^{+3} was used to trace the penetration of marinade in chicken breast meat.

Results showed that increasing concentration of salt and pH in marinade increased the persistence of the pink color in cooked meat. Furthermore, degree of denaturation of myoglobin pigments was not a determining factor for cooked meat color under the condition of higher ORP values in meat products. On the other hand, temperature of marination was found to be a significant factor in marinade penetration and retention in a vacuum tumbling process. Higher

marination temperature promoted deeper penetration of marinade in the meat as traced by trivalent Eu^{+3}. Consequently, marinade pick-up was found at higher temperature. However, cook yield was found highest when marination process was initially at a higher temperature followed by lowering to near refrigeration temperature. Amount of salt soluble protein extracted at lower temperature was higher compared to higher temperature of marination that may be a factor in higher marinade retention on cooking

In conclusion, pink color incidence in cooked meat is dependent to the ORP value of meat during and after cooking. Pink color can be induced in a product that has the cooked meat color by altering the ORP by addition of a reducing agent. In addition, Eu^{+3} tracing of marinade penetration in meat showed marinade to be only near the surface of the chicken breast meat even at the elevated temperature of vacuum tumbling.

INDEX WORDS: Fluorescence, marination, vacuum tumbling, pinking, cook yield, expressible moisture, water retention, europium

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EDWIN YPARRAGUIRRE PALANG

B.S., University of Santo Tomas, Philippines, 1995

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EDWIN YPARRAGUIRRE PALANG

Major Professor: Romeo T. Toledo

Committee: Mark Farmer
Yao-wen Huang
William Kerr
Estes Reynolds

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

I would like to dedicate this work….

……to my parents Jeana and Pedro Palang. Their unwavering support and all encompassing love have kept me going during the long pursuit of this degree.

……to my supportive siblings Manong Edmund, Manang Ruth, Doin, and Jeanette, who are unselfishly proud of their brother's accomplishments.

…….to my aunt Lily, Uncle Nof and my cousin Aileen for opening their home to me. Their kindness and open-mindedness are exemplary and worthy of envy.

……to my dear friends Adam and Maricel, who were part of this undertaking.

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TABLE OF CONTENTS

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Long before its use in commercial meat processing operations, marination technology had been utilized in domestic meat cookery. Webster's dictionary defines "marinade" as a pickle, and marination implies pickling or soaking meat in a marinade solution for hours. This was commonly done in domestic kitchens, but to accelerate marinade absorption by meat, commercial marinades were injected, tumbled or massaged using mechanical systems. Marination technology has expanded from poultry meats to red meats and fisheries products. Furthermore, the objective of marination has expanded from flavoring meats to tenderization and improvement of cooked meat juiciness.

Indices of successful marination consists of: complete pickup by meat of all added marinade, avoidance of liquid separation from the raw meat (purge) and the retention of marinade on cooking to maximize cooked yield. Meats with high cooked yields are generally tender and juicy. These desirable characteristics of a successfully marinated meat are attained by the interaction of marinade ingredients with the meat matrix. It is generally assumed that salt soluble proteins are extracted. Actomyosin is broken down to actin and myosin, and functional ingredients such as phosphate opens up spaces within the protein matrix to trap water. Marinade ingredient functionality is maximized only when the marinade is uniformly incorporated into the meat. Otherwise, marinade ingredient and meat interaction will be localized if the marinade is only found at or near the surface of the meat. In addition, marinade should be well distributed in the meat as mechanical action such as tumbling or massaging is applied to promote the functional ingredient interaction. Furthermore, on cooking, the red or pink color of raw meat

should no longer be present otherwise consumers may judge the meat as undercooked. Today's consumers are knowledgeable about food safety issues and the hazards of consuming raw pork or poultry is well known to most consumers.

This study was conducted with the following objectives: (1) To determine the factors affecting the meat pigments rate of thermal denaturation and to relate extent of meat pigment denaturation to elimination of the red or pink color; (2) to optimize temperature employed in the vacuum tumbling process to maximize marinade uptake and retention, and (3) to investigate the use of a low molecular weight compound to track marinade diffusion into meat. Simultaneous studies on meat pigment color in heated meats and marinade retention are needed because literature data indicate that the conditions which favor meat pigment stability are also conditions which favor marinade retention. These studies will elucidate the relative incongruity of maximizing marinade retention and prevention of the pink color defect in cooked chicken white meat.

Consumers' Perspective of Cooked Meat Color

Simply put, meat color leaves a powerful visual impression with the consumer, (as influenced by the structure and texture of the meat muscle when viewed) (Renerre, 1990). The decision by the consumer to purchase, or conversely to forgo, a meat item is based in part on the color of the product in question. In raw meat, a color that is either too pale or too dark is frowned upon. Consumers prefer normal colored meat (Topel et al. 1976; Wachholz et al. 1978). For raw red meats, the deep red color is associated with freshness, and thus, is preferred by the consumers. On the other hand, brown color of raw meat is considered to be undesirable by the consumers (Gorelik and Kanner 2001; Renerre 1990). Ironically, the brown color is strongly

desired by consumers in cooked uncured meat products (Egbert et al. 1986). Additionally, consumers will perceive a pink colored cooked meat as undercooked.

The USDA specifies different endpoint temperatures for different meat products for safety purposes. Monitoring the internal temperature of cooked meat is the recommended means to ensure that the meat has reached the recommended end point temperatures. However, for consumers, the cooking endpoint is judged by visual evaluation of internal color and appearance. The release of clear meat juices from cooked meat while standing on a counter after removal from the oven or grill, is also associated with the meat doneness. In many instances, the internal color of cooked meat may still be pink, even when the recommended end point temperature has been achieved. When meat undergoes premature browning on the outside, the interior may still be raw (red or pink) although the meat appears to be cooked on the outside. In general, the pink color usually disappears in meat even before the safe end point temperatures is reached (Lyon et al. 2000).

The occurrence of cooked white meat having the pink color is a growing concern in the food industry as more Ready-to-Eat (RTE) meat products are produced. RTE products may be rejected by consumers when the meat appears pink since consumers may think the product is undercooked (Maga 1994).

Factors Affecting Concentration of Pigments in Meat

Several factors affect pigment concentration in meat. Intrinsic factors such as the muscle type, animal, age, breed, sex, diet, etc. are important sources of variation in the concentration of meat pigment (Fletcher 2002). Furthermore, extrinsic factors such as pre-slaughter history, chilling mode, electrical stimulation, hot-boning, etc., might affect the conversion of the myoglobin pigment into several derivative forms.

Muscles often used by the animal for movement (locomotive) contain more myoglobin than the stationary muscles. In poultry, the concentration of myoglobin is higher (0.21 mg/g) in leg muscles than (0.15 mg/g) in the breast (Froning 1995). Myoglobin constitutes 80 to 90 percent of the total pigment on a well-bled muscle tissue (Hendric et al. 1993). Besides the skeletal muscles, cardiac and smooth muscles are also rich in myoglobin. The heart has about 4.0 mg/g tissue and the gizzard about 20.0 mg/g tissue (Nishida 1989). Furthermore, older animals contain higher myoglobin levels than young animals. A myoglobin concentration of 0.01 mg/g white meat was found in 8 week old poultry while 0.1 mg/g white meat was found in 26 week old male poultry (Fletcher 2002). Cytochrome c is found in much lower levels compared to myoglobin. In chicken breast only about 0.011 g/g tissue of cytochrome c was found. Furthermore, only about 0.035 g/ g tissue of cytochrome c was found in the chicken thigh (Pikul et al. 1986). In addition, while cytochrome c was constant at 2.5% of heme pigments in chicken breast, the percentage varied in thigh muscles (Pikul et al. 1986). Other pigments in meat that are present minimally are vitamin B_{12} and the flavins (Pegg and Shahidi 1997).

Muscle to muscle differences in myoglobin content depends on the type of muscle fibers. Muscle fibers with a predominantly oxidative (aerobic) metabolism have higher myoglobin content compared to muscle fibers with a mainly glycolytic (anaerobic) metabolism (Warriss 2000). The dominant fiber type in the muscle determines the muscles' macroscopic color. Slow contracting fibers appear redder in color than the fast contracting fibers, with the latter being the dominant form in the white muscle (Nishida 1989).

Chemistry of Myoglobin Derivatives

The myoglobin molecule is comprised of globin protein that surrounds the protoheme (proto porphyrin IX), a large planar ring. Methene bridges connect four pyrrole rings that

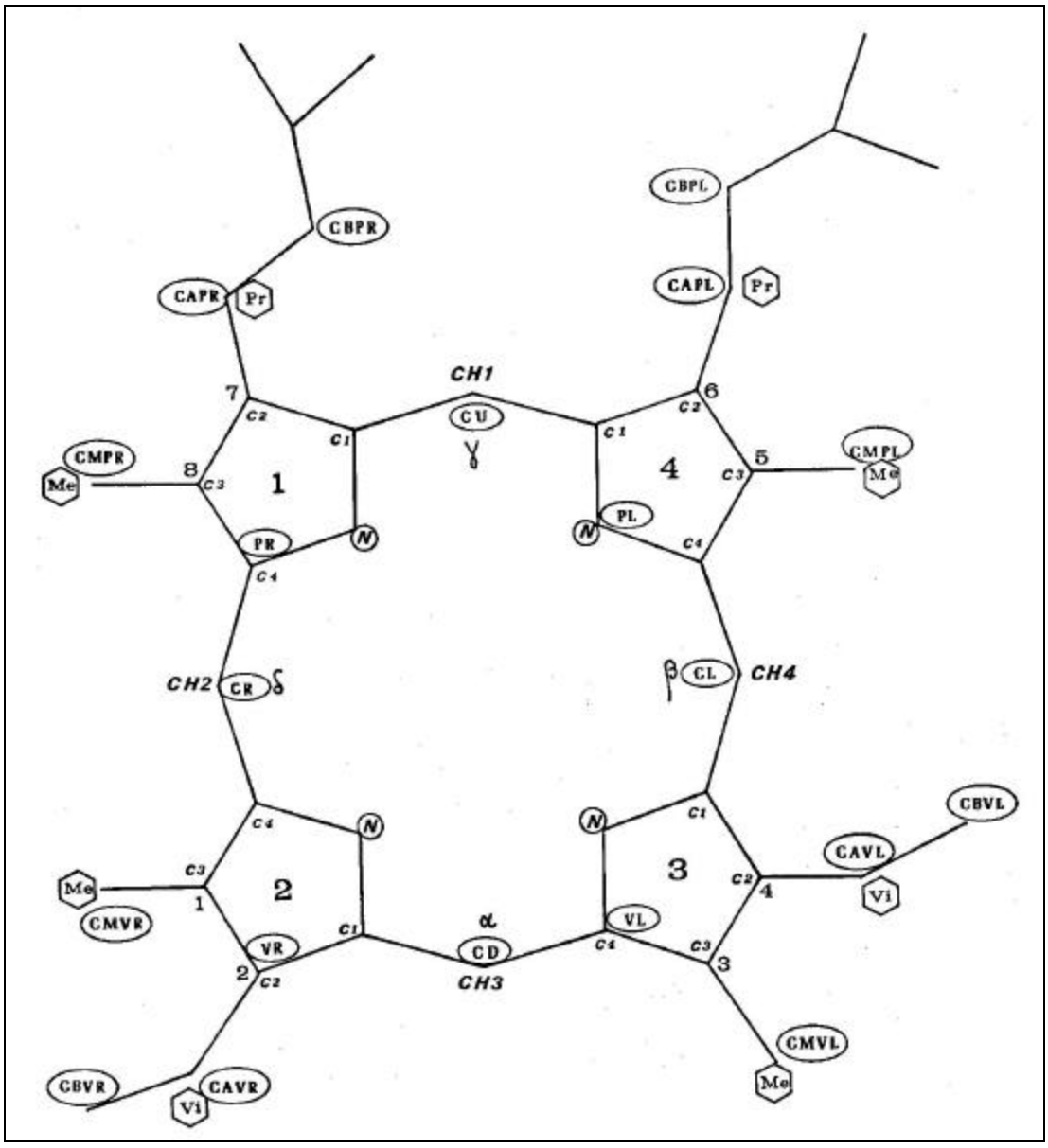

Figure 1.1. Structural illustration of protoporphyrin IX of myoglobin (adapted from Antonini and Brunori 1971).

comprise myoglobin. Furthermore, in the external positions 1-8 from Figure 1.1, different groups can attach. The variation of substituents attached in positions 1-8, gives different chemical forms that differ in solubility, light absorption or reflectance spectra and reactivity (Antonini and Brunori 1971). The iron atom is found in the central position of the porphyrin ring. In the fifth and sixth positions, iron forms an octahedral complex upon coordination with different ligands. The sites from 1-4 are bound to four pyrroles. Side chains containing amino acids are in contact with the heme group as well.

Myoglobin has a molecular weight from 16,000 to 18,000 and has extinction coefficient of 11.3 mM/L (Rickansrud and Henrickson 1967). There are six ligands binding sites about the iron atom, four of which are occupied by the pyrrole nitrogen atoms of the porphyrin ring. The fifth ligand is linked to the nitrogen atom of an imidazole of a histidine residue. The sixth site is free for ligand binding.

The iron oxidation state of the heme compound determines which ligand can attach to the sixth ligand. Ligands that can bind are nitrate/nitrite, CO, water and oxygen. Water and oxygen are endogenous to the meat while the nitrite and CO are acquired externally or from contamination.

The heme in myoglobin is embedded in a cleft made by the polypeptide chain near the surface of the molecule. Globin protects the iron from oxidation (Ladikos et al. 1988). The heme iron interacts with the imidazole of histidine F8. The heme group is bound to the polypeptide chains by non-covalent bonds. The affinity of heme for the protein at neutral pH is very high. It involves complex molecular interactions between the heme and the protein and also involves about 90 Van de Waals contacts (Ladikos et al. 1988). Dissociation of the heme is increased considerably at acid pH.

There are three different chemical states of myoglobin derivatives in meat. Myoglobin derivatives are constantly interconverted in meat tissues depending on the ligand attached to the free binding ligand site. They are deoxymyoglobin, oxymyoglobin and metmyoglobin (Fig. 1.2).

Deoxymyoglobin

The chemical state of myoglobin is deoxymyoglobin when the iron heme compound is in ferrous form with no ligand bound to the sixth ligand site. Deoxymyoglobin is the color found in the interior tissue of meat, and will persist in this form as long as reductants generated within the cells by enzyme activity are available (Pegg and Shahidi 1997). Deoxymyoglobin can be formed from oxymyoglobin under vacuum or inert equilibration. The addition of reducing agent to the metmyoglobin can make the pigment revert back to deoxymyoglobin in absence of oxygen. Among the chemical reagents used to form deoxymyoglobin are dithionite, ferrous salts (ferrous pyrophosphate) sodium borohydride, and ascorbic acid. Enzyme systems can also reduce ferric to ferrous heme in the presence of an inorganic catalyst and an oxidation-reduction mediator (Antonini and Brunori 1971). Solution of deoxymyoglobin has a typical red violet color which becomes greenish at high dilutions.

Factors that affect the oxidation of myoglobin include pH, salt type and concentration. According to Wallace et al. (1982), salt such as sodium chloride increase the oxidation rate of purified bovine myoglobin in direct proportion to the anion (chloride) concentration. The oxidation reaction converts deoxymyoglobin to metmyoglobin. Observations made on meat pigments in solution may not be the same in a meat system since other chemical reactions in the meat can affect the autoxidation rate and the enzymatic reduction rate of myoglobin. The rate at which meat or meat products discolor cannot be predicted based solely on results obtained with

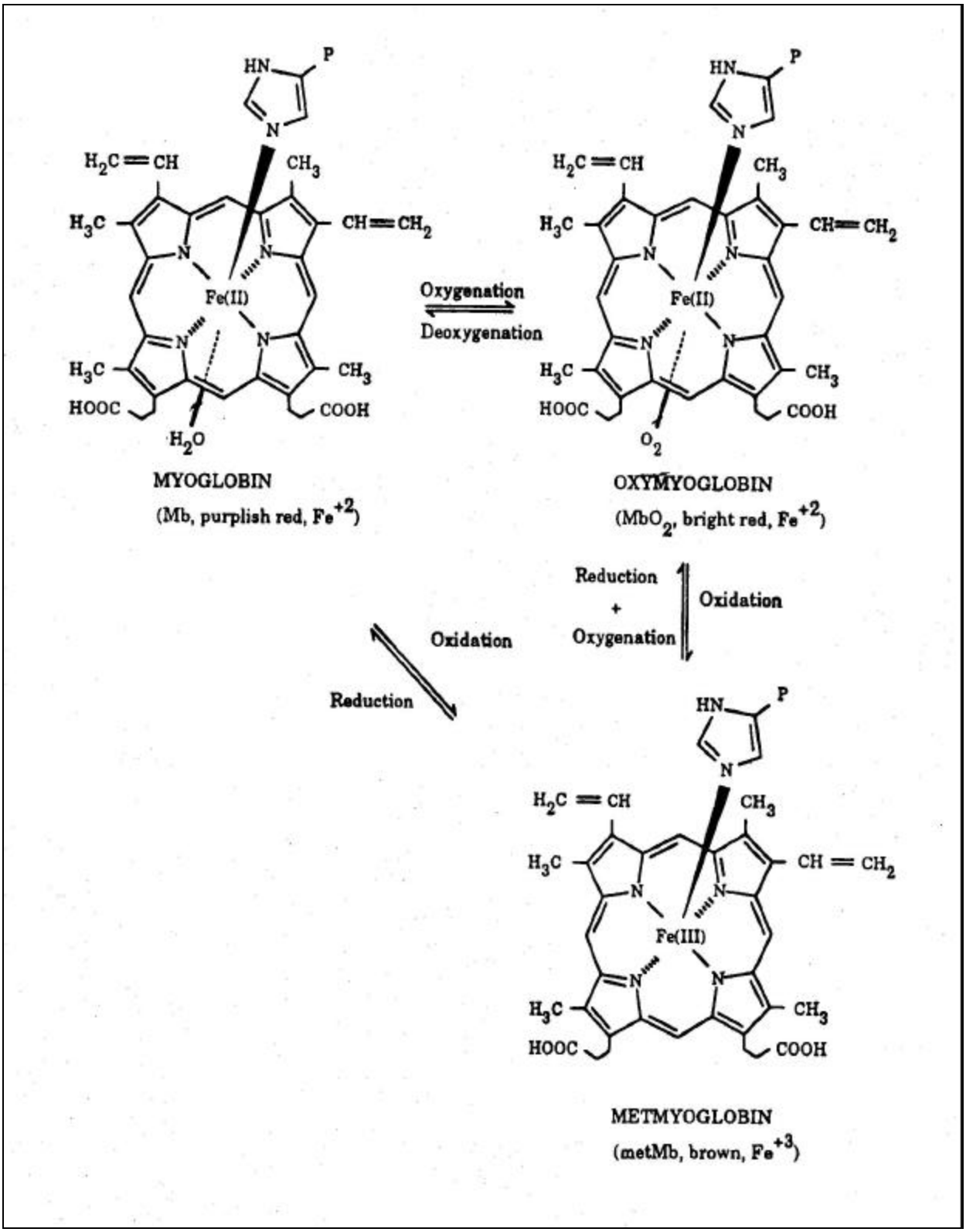

Figure.1.2. Illustration of myoglobin interconversion (adapted from Pegg and Shahidi 1997).

purified myoglobin (Trout 1990). The myoglobin visible spectrum was observed to have a large absorption peak at 550 nm (Antonini and Brunori 1971).

Oxymyoglobin

Deoxymyoglobin when exposed to air binds molecular oxygen to the available sixth ligand binding site converting deoxymyoglobin to oxymyoglobin. The iron heme compound is still in the reduced form. One mole of oxygen is bound per mole of ferrous heme iron. This reaction of oxygen removal from the sixth ligand binding site to convert oxygenated to deoxygenated myoglobin is completely reversible. Oxymyoglobin is not infinitely stable. Autoxidation could occur and the ferrous heme compound is converted into ferric heme compound, metmyoglobin. In the presence of a reductant, metmyoglobin can be converted to oxymyoglobin. Visible spectra of oxymyoglobin exhibited absorption maxima at 540 and 577 nm (Antonini and Brunori 1971).

Metmyoglobin

Autoxidation converts oxymyoglobin to metmyoglobin. Low oxygen partial pressure can favor the formation of metmyoglobin as well. The rate of autoxidation is enhanced at higher temperatures, low partial pressures and high salt concentration (Antonini and Brunori 1971). The maximum rate of metmyoglobin formation has been reported to occur at oxygen partial pressures of 6-7.5 mmHg and is dependent on pH and temperature (Renerre 1990). Addition of ferric cyanide can result in the oxidation of the iron heme compound of myoglobin from ferrous to ferric. Water binds to the sixth binding site in the oxidized iron heme compound, and a dark brown color is exhibited in highly concentrated solutions while color is yellow-green in dilute solution under acidic condition (Atonini and Brunori 1971). Once myoglobin is oxidized to

metmyoglobin, metmyoglobin remains stable. Metmyoglobin can not revert back to deoxymyoglobin or oxymyoglobin, unless the iron heme is reduced first. A reducing agent has to be added to the metmyoglobin to reduce the Fe in the heme to obtain the chemical form of deoxymyoglobin or oxymyoglobin. Ascorbic acid can prevent metmyoglobin formation by acting as an oxygen scavenger (Renerre 1990). Metmyoglobin can be enzymatically reduced by a metmyoglobin reductase with NADH as coenzyme into oxymyoglobin or deoxymyoglobin (Osborn et al. 2003).

Acidic metmyoglobin spectra shows two bands in the visible wavelength with maxima at about 500 and 635 nm; the Soret band lies at about 405nm. The alkaline form has two bands in the visible wavelength with maxima at about 540 and 580nm; and therefore, is more reddish in color. The Soret band has usually a much lower extinction coefficient than that of the acid form, and a maximum at ~412nm (Antonini and Brunori 1971).

Color of Raw Meat

Myoglobin is found in the smooth or striated muscles of all animals. Myoglobin exists in different chemical states depending on the ligand attached to its free ligand binding site. The color imparted by these derivative compounds can be desirable or undesirable depending on the meat product desired.

The freshly cut surface of meat is purple because the pigment is in the deoxygenated form. Upon exposure to air, the myoglobin at the surface, and to a depth of 2-6 mm (or more) reacts with oxygen to from the desirable bright red oxymyoglobin. This process takes about 15 minutes to one hour (Warris 2000). The chemical reaction which changes reduced myoglobin from a deoxygenated to oxygenated form is known as blooming. The depth of the oxymyoglobin layer depends on the extent of penetration of oxygen from the atmosphere. According to

Ledward (1970), in between the layer of oxymyoglobin (near muscle surface) and deoxymyoglobin (interior of muscle), a very thin layer of oxidized metmyoglobin forms due to the low partial pressure of oxygen present. Furthermore, the same author found the maximal formation of metmyoglobin formation at partial pressures of oxygen between 6 and 7 mm in beef semitendinosus muscle stored between 0 and 7^{o}C. Metmyoglobin can only by reduced to a limited extent, persists in the oxidized form particularly in meat that has been aged for a long time post mortem and in which the reducing activity of the muscle enzymes is consequently low (Warris 2000).

Oxymyoglobin is more resistant to oxidation than the deoxygenated myoglobin. The depth of the oxymyoglobin layer varies slightly between muscles because of their different metabolic characteristics. This is particularly true with the activity of the various enzyme systems which continue to be active for a time after the death of the animal. The oxymyoglobin layer is thinner in muscles with high activities of reducing enzyme systems, which also continue to be active for a time after death of the animal. However, the layer is thinner in muscles with high activities of reducing system enzymes, particularly the cytochromes. The reducing activity of enzymes decreases with age, post-mortem. Furthermore, reducing activity decreases with temperature and is less affected by oxygen diffusion through tissue. The thickness of the oxymyoglobin layer is therefore greater, and meat color is brighter, at lower temperatures of storage.

After about 2 or 3 days exposure under ambient condition, the oxymyoglobin found on the meat surface gradually starts to oxidize to metmyoglobin. When around 20% of the surface pigment has oxidized, the change in color of the meat can be enough for consumers to discriminate on color (Warriss 2000). The same author found there is a difference between

storage of meat at 0^oC compared with 5^oC, browning of the surface is delayed by around 48h to 1 week.

Non-Native Forms of Myoglobin Pigments

The free binding site of the globin is not only limited to the binding of oxygen or water, but it can bind as well to other compounds such as nitrites, cyanide, sulfur and carbon monoxide. Furthermore, linkages can be destroyed by high oxidizing agents such as the peroxides. These compounds are not endogenous to the meat but introduced externally. These compounds are contaminants from water, air, marinade ingredients, and other processing procedures.

Nitric oxide, when it reacts with reduced myoglobin, gives meat a bright red color or a pink color as in cured meats. Furthermore, carbon monoxide, when it reacts with reduced myoglobin, imparts a red color and forms carboxymyoglobin. Enough low levels of sodium nitrite (6 ppm) in chilled ice were found to demonstrate a pink color in cooked chicken leg and thigh meat (Nash et al. 1985). Ahn and Maurer (1985) found levels of nitrite as low as 1 ppm to be sufficient to give a pink color in turkey breast. Similarly Heaton et al. (2000) found 1ppm of sodium nitrite to be enough to induce pinking in chicken breast. Discrimination of ligand binding to CO and NO over O_2 is based on the electrostatic interaction with the bound ligand and solvent water molecules on the distal side of the heme group (Olson and Phillips 1997).

Metmyoglobin have derivative compounds called cyan-myoglobin formed by the addition of cyanide to ferric myoglobin. Cyanide displaces water from the sixth ligand binding site. Cyanide ferric derivatives are very stable but react directly with dithionite to reduce the heme iron while the cyanide is still bound. Ferric becomes ferrocyanide derivative which then dissociates into cyanide and deoxygenated ferrous heme protein (Antonini and Brunori 1971). In comparison to other derivatives, cyanide ferric derivatives are resistant to denaturation.

Denatured metmyoglobin gives off a brown color. However, when further oxidized by bacterial action or photochemical oxidation, denatured metmyoglobin can be transformed to either colorless or yellow (Pegg and Shahidi 1997). The dissociation of oxidized denatured globin hemichrome was found to impart green color (Cornforth et al. 1986).

Ahn and Maurer (1990) found that pyridine, albumin, bovine serum albumin, and gammaglobulin react with each other, the result being a complex formation with myoglobin that in turn had a color ranging from orange-red to pink.

Hemoglobin

Hemoglobin is 4 times bigger than the myoglobin molecule. Hemoglobin is composed of two different types of polypeptide chains, which have been called a and ß chains. The reaction mechanism of hemoglobin is similar to myoglobin but varies in reaction rates. Hemoglobin derivatives occur as oxyhemoglobin, deoxyhemoglobin and methemoglobin are similarly formed by attachment of ligand to the sixth ligand binding site as dictated by the charge of iron heme compound. Hemoglobin derivatives are formed with the similar reaction mechanism as the myoglobin derivatives.

Cytochrome c

Cytochrome c is a heme protein with a molecular weight of 13,000. Cytochrome c transports electrons and is found in the mitochondria. It has absorbance peaks at 415, 520 and 550 nm (Ahn and Maurer 1989). In dark muscle meat concentration of cytochrome c is comparatively much lower compared to myoglobin. In white meat cytochrome c concentration can be comparatively high since myoglobin concentration is lower. In turkey, concentration of myoglobin was found to 0.58mg/g and the concentration of cytochrome c was 0.013 mg/g (Ahn

and Maurer 1989). Denaturation of protein in cytochrome was found to be more heat stable than the myoglobin (Girard et al. 1990; Ahn and Maurer 1989). According to Ahn and Maurer (1990), cytochrome c may be a source of heme in formation of denatured globin hemochromes which complex well with nicotinamide or histidine. The reduced cytochrome c has a pink color while the oxidized form is yellow (Pikul et al. 1986).

Causes of Color Abnormalities in Meat

Unusual color developments may occur in meat in several ways, some of which are unrelated to normal chemical reactions of the pigments. The Pale Soft Exudative (PSE) and dark cutting condition in meat are partially the result of unusual degrees of water binding in the muscles, and consequent alteration of light reflection. PSE is caused by the fast pH drop during post-mortem glycolysis (Lindahl et al. 2001). The paleness of PSE meat is caused largely by a high proportion of free water in the tissues, which is located between the muscle cells rather than within them. Tissues containing a great amount of extracellular water have many reflection surfaces that totally reflect light, but have a limited light absorption capability (Briskey 1964; Lindahl et al. 2001). Color intensity therefore is greatly reduced. In dark cutting meat, high water-binding capacity maintains an unusually large proportion of water as intracellular water. Because of this, white light reflection is minimized and color absorption is enhanced. Dark cutting tissue also has a high rate of oxygen-using enzyme activity, due to its high pH. This reduces the proportion of the pigment in the red oxygenated state.

Dry firm dark (DFD) meat is caused by glycogen depletion ante mortem, and is therefore characterized by much lower levels of carbohydrates in the muscle (Warris 2000). DFD looks dull and purpler than normal meat while PSE meat has a more yellowish color. High pH results in relatively little denaturation of the proteins, water is tightly bound, and little or no exudate is

formed (van Laak1999). There is little or no shrinkage of the myofilament lattice and the differences in refractive index of the myofibrils and sarcoplasm are reduced. The muscle presents a closed, translucent structure that absorbs rather than reflects light. This makes the meat appear dark. The close structure reduces the diffusion of oxygen into the muscle from the surface and any oxygen that does reach the interior is used up by the high cytochrome activity encouraged by the high pH. This results in only a very thin surface layer of bright red oxygenated myoglobin allowing the purple color of the underlying reduced myoglobin to show through (Renerre 1990).

Freezing rate also affects color of meat. Slowly frozen meat is excessively dark while meat frozen in liquid nitrogen is unnaturally pale (Renerre 1990). The faster the freezing rate, the lighter the product becomes due to the small ice crystals formed by fast freezing that scatter more light than large crystals. If frozen meat is stored in the dark, the attractive color lasts many months. It has been shown, for example, that at $-18^{o}C$, the color remained attractive for 2 months in the dark but only 3 days when exposed to light (Rennere 1990). Photo-oxidation of the pigment is a problem in meat storage and is dependent to muscle type.

Iridescence is the 'rainbow-like' discoloration occasionally found on fresh meat but very common in cooked meat products due to the fibrous character on the meat surface caused by the 'prism effect' (Renerre 1990). This is commonly observed in cooked pork, turkey and beef.

Cholеglobin is a green pigment induced by high oxidizing agents such as hydrogen peroxide reacting with the heme. Sulphmyoglobin is a derivative of myoglobin that gives green color. Sulphmyoglobin is formed when the heme compound in myoglobin reacts with sulfur compounds (Walters 1975).

Disappearance of Pink Color in Cooked Meat

High heating temperature denatures the globin protein of both myoglobin and hemoglobin compounds. Further heating may result in the break down of bridges between the myoglobin heme compound and the globular protein. Stearic hindrance by the iron of the heme compound will be limited since the globular protein denaturation draws the amino side chains away from the heme compound. Iron is then exposed to the oxidant and the free ligand binding site becomes open to ligands such as water to impart a brown color or to nicotamide to impart red color during heating (Maga 1994).

The mechanism of globular protein denaturation of myoglobin was suggested by Awad and Deranleau (1968). The first phase leading to complete denaturation is conformational disturbance in the region of the heme group, followed by the unfolding of the helical regions in the molecule and finally a sequence of aggregation occurs, leading to the precipitation of the globular protein. Denaturation of myoglobin may play an important role in the disappearance of the pink or red color in cooked meat. During denaturation, the heme compound is exposed and easily accessible to ligands and oxidants.

Chen et al. (1984) found that the optimum temperature for iron to be released from heme is between 62 and 73^{o}C. According to Han et al. (1993), most of the heme moiety remained still intact even at 100^{o}C. No literature was found involving the study of meat color associated with the dissociated form of myoglobin pigment. Mendenhall (1989) showed that formation of ferrichemochrome takes a longer time at higher pH or higher cooking temperature. Ferrichemochrome is the converted form of deoxymyoglobin, oxymyoglobin, and metmyoglobin pigments when denatured.

The external meat color of cut meat when heated with dry heat is a result from a combination of surface dehydration and sugar amine browning reactions. Amine groups on muscle proteins react with any available reducing sugars, such as free glucose, in tissues. Browning occurs at high (approximately 90^{o}C) temperatures, such as those found at cut surfaces during roasting or broiling. Cooked meat pigments show the brown color of metmyoglobin because of oxidation and denaturation of globular protein from heat (Hedric et al. 1993).

Other studies considered the binding of alternative ligands such that ligands which give red color and showed these ligands will not get attached to the myoglobin. In an attempt to find a solution to the pinking problem, Schwarz et al. (1997) found diethylenetriamine pentaacetic acid, ethylenedinitrilo-tetraccectic acid disodium salt, 1,2-diaminiocyclohexame-N,N,N',N' tetraacetic acid monohydrate and calcium reduced non fat dried milk to be effective in reducing pinking in products with added nicotinamide or nitrite. The addition of 3% dried milk solids prevented the formation of pink discoloration in turkey rolls (Dobson and Cornforth 1992). Citric acid was also found to reduce pink color in cooked ground turkey (Samuel et al. 2003). Furthermore, the use of 2% sodium lactate was found to eliminate discoloration when vacuum packaged bratwurst was cooked at 74^{o}C (Ghorpade et al. 1992). Browning agents like Maillose, which are commercially available is used for meat product applications as an intervention to pink color disappearance in meat

Pink Color Persistence in Cooked Meat

The presence of pink color in cooked meat was postulated to be due to the undenatured protein moiety of myoglobin. Furthermore, due to stearic hindrances from the globular protein that envelope the heme compound, Fe is not readily available to oxidizing agents. Trout (1989) stated the color of cooked meat may be related to, at least in part, to the undenatured native

pigments of myoglobin, hemoglobin, and cytochrome c. Ghorphade and Cornforth (1993) found pink color present in roasted pork at 65^{o}C and observed undenatured myoglobin in beef. However, pink color has been observed in cooked meat products that have been cooked at end point cooking temperatures exceeding protein denaturation.

Myoglobin affinity to ligands like oxygen, carbon monoxide, and nitrite/nitrate could result in a strong red color (Schwarz et al. 1997; Geilesky et al. 1998; Holownia et al 2004). Ahn and Maurer (1990) also observed myoglobin, hemoglobin and cytochrome c could form a complex with histidine, cystine, and methionine, or with the side chains of solubilized proteins and vitamin B_6 derivatives which are native to the meat. Its color ranged from orange to pink, suggesting another important factor in the pink color of cooked meat.

The change in oxidation-reduction potential of cooked meat has been considered as a major factor on persistence of the pink color in cooked meat. At high temperature, measured ORP has negative values. Sodium chloride and phosphate were found to play a significant role in pink color intensity in cooked meat. NaCl and phosphate in solution resulted in a decrease in ORP value (Ahn and Maurer 1989). Furthermore, chloride ions was said to cause pink color. Du et al. (2002) found irradiated meat has a low ORP value which may reduce metmyoglobin to myoglobin and keep the iron in reduced form. Nam et al (2002) found the decreased ORP from irradiation also produced gaseous compounds that can act as a sixth ligand of myoglobin, such as carbon monoxide. Katayama et al. (1992) found radiolytic carbon monoxide in irradiated meats. Enzymes naturally found in meat like metmyoglobin reductase can maintain reducing conditions in meat during cooking, thus metmyoglobin formation is hindered and oxymyoglobin is instead formed (Osborn et al. 2003).

Metmyoglobin and ferrichemochrome could be reduced to form oxymyoglobin pigment under reducing conditions with either enzymatic or non-enzymatic reactions (Osborn et al. 2003). The enzymatic reaction involves the use of metmyoglobin reductase with NADH. Also, ascorbate in the presence of oxygen gas could reduce metmyoglobin to oxymyoglobin (Tsukahara and Yamamoto 1983).

The increase of pH results in a decrease in lightness, and an increase in pink color in cooked meat (Brewer et al. 2001). Cooking of high pH beef (pH>6) compared to lower pH (5.5) was found to be redder and appeared undercooked (Gasperlin et al. 1997). A positive correlation was found between pH and visual pink color (Brewer et al. 2001). The increase of pink color intensity in meat could be due to the reduction of Fe since higher pH favors the existence of ferrous iron (Maga 1994). Normal meat with a pH between 5.3-5.7 was found to favor the production of ferrichemochrome, the grey pigment of cooked meat (Mendenhall 1989). In addition, high pH stabilized globular protein as shown by the decrease in the amount of myoglobin denatured. Also, pink hemochrome was observed when meat is heated to a temperature more than 76^{o}C (Trout 1989; Hunt et al. 1999).

Under the assumption that pH, salt, and phosphate influences the pink color in cooked meat, marination technology should be carefully studied since it can effectively change the meat pH, ionic strength, and ORP conditions. Salt and phosphate are the principal ingredients of marinade which are incorporated in meat to improve the water holding capacity as well as the texture of the meat. Currently, more marinated meat products, including fresh whole muscle meat, are being sold in the market. An optimum condition of marinade formulation with respect to pink color, water holding capacity and cook yield would be a good opportunity for research leading to a clearer understanding of the key role played by primary marinade ingredients.

Pinkness Intensity Measurement

The color spectrum of meat can be measured and expressed into spatial spaces. Hue describes color as yellow, green, blue or red while chroma describes the intensity of a fundamental color with respect to the amount of white light mixed with it, and the L^* value is an indication of overall light reflectance (brightness of the color).

There are different systems used to measure color with the aid of an optical instrument. Objective measurements of color are expressed in CIE L*, a* and b*, Hunter Lab CIE L*,u* and v*, and xyY systems (Garcia-Esteban et al. 2003). Visual inspection is also practiced to discriminate color differences. However, human visual judgment could be influenced by the source of light and the angle the light hits the surface of the object. Simply put, visual judgment can be subjective. Instrumental measurement is widely practiced because it is reliable, inexpensive and easy to use. Furthermore, results are more easily reproduced than with visual inspection. However, instrumental measurements will not mean anything unless the measured values are incorporated into the human perception of an acceptable color range. Human visual sensitivity is not linear to instrumental measurements between wavelength, calculated color measure, and human sensitivity. Thus, in this case, human perception is essential in drawing a threshold level between the cooked and uncooked color of meat (Brewer et al. 2001).

In this study, CIE L*, a*, and b* values were used. The CIE L*a*b* system was found to correlate best to sensory, visual and objective assessments of color (Garcia-Esteban et al. 2003) as proven by Ferreira et al. (1994) using illuminant C as source of light. L* measures the lightness of meat. Lightness is dependent to the scattering of light upon the surface of the meat. High L* value represents a whiter or paler color while the lower L* value means a darker meat color. According to Brewer et al. (2001), high pH meat has dark color, which minimizes the

scattering of the light due to the unavailable free water on the meat surface. Furthermore, he stated that the water in meat is bound by protein at a higher pH, and thus light scattering is limited.

The positive a* value of the color space represents redness and positive a* value the blue color. L and a* value are associated in the perception or measurement of pinkness or redness. The higher a* value represents a more red color. The high L* and low a* value correlate closest to the true red color (Breyer et al. 2001). The other value of the color space is the b*, which measure the yellowness of the color spectrum. Hue angle is also an important measure for pinkness intensity indication, but it is only secondary to L* and a* (Brewer et al. 2001).

Marination Technology

Marination is employed to meat to improve texture, water holding capacity and cook yield of meat. Other uses of marination are to improve product shelf life and to make value-added products. There are different formulations used for marination but the principal ingredients are phosphate, salt and water. Antimicrobial agents and flavoring are secondary ingredients in marination. The level of concentration of each primary ingredient depends on the product desired. The different formulations used for marination are a potential source of color problems issues since, depending on the marinade, meat conditions can be significantly altered.

Commercial Marination

The common methods of marination used in the food industry are vacuum tumbling, massaging and injection. In some marination operations, injection and vacuum tumbling are both used to marinate the meat.

Automated marination systems, particularly with boneless meats, employ vacuum and mechanical action by tumbling or massaging. Tumbling imparts mechanical action by lifting the meat to the highest point in the rotation of a cylindrical drum and dropping the meat to the lower surface by gravity. The mechanical action is strongly a function of the relative quantity of a load within a particular drum size. Massagers, on the other hand, utilize a stationary drum equipped with internal paddles. Mechanical action is generated by the alternate squeezing and relaxation of the meat as the paddles force the pieces of meat against each other. Conventionally, marination is performed at refrigeration temperatures. Isothermal marination is practiced due to the limited capability of commercial tumblers to operate at different marination temperatures. It is only recently that massagers allow for the marination operation to be conducted at different temperatures. In both vacuum massaging and tumbling, the size of meat load relative to the size of the unit, the marinade to meat ratio, the length of a marination cycle, the speed of the drum rotation or agitator speed, the vacuum and the temperature are important parameters. Marinade is infused into the meat faster with the mechanical action, as compared to a static soaking process. Tumbling can also be carried out in a vacuum. This is essential for the marination to allow the penetration of the marinade into the interstitial spaces of the meat muscle more easily. Marinade diffuses faster into the internal part of the meat as a result. Marinade absorption has been observed to be higher under vacuum marination and tumbled when compared to static and atmospheric marination (Chen 1980). Tumbling operations can vary from 15 to 30 min. depending on the muscle type and percent marinade absorption desired. Tumbling marination time is limited by the adverse effect on the texture of the meat. Excessive tumbling could result in extraction of meat proteins, and in turn give marinated meat a rubbery texture. There was no extensive study of temperature optimization in marination by tumbling or

massaging found. A benefit found in marination at higher temperatures by Fenton et al. (1993) was that marination at room temperature resulted in more tender meat than under refrigeration temperature. Heath and Owens (1991) saw an advantage processing at sub-ambient temperature by slowing the bacterial growth, preserving ingredient function properties, and the salt soluble proteins solubility was higher.

Bone-in meat products are mostly marinated by injection. Meat products pass through an array of needles and the marinade is injected into the meat matrix. Marinade is delivered and distributed into the meat under pressure. The speed of the conveyor is essential to the amount of marinade injected to the meat. Furthermore, the orientation of the needle is also important in attaining homogeneous distribution of the marinade in the meat. Injection and tumbling marination methods are used as a two step marination process. Marinade is first injected into the meat and followed by tumbling to help evenly distribute the marinade into the meat. Furthermore, the mechanical tumbling action also assists with the increased interaction of the salt soluble protein and the marinade ingredients. The process may improve the water holding capacity and cook yield of meat due to an increase of protein and marinade interactions.

Marination Ingredients Functionality

Primary ingredients in marinade are salt, phosphate, and water. The concentration of each ingredient in the marinade formulation can vary according to the product desired. The maximum phosphate concentration allowed in meat is capped at 0.5 percent by the United States Department of Agriculture (USDA) (9CFR318). Furthermore, secondary ingredients such as flavor essence, antimicrobial agents, and starches can be added to improve shelf life, water holding capacity and add flavor in meat (Palang and Toledo 2001).

The primary ingredients sodium chloride and phosphate are mainly added to improve the water holding capacity, cooking yield and tenderness of meat (Xiong and Kupski 1999a). Another beneficial functionality of phosphate is to improve the oxidative stability of meat (Ang and Young 1987). Meat conditions could vary depending on the pre and post slaughter stress induced in meat, and this has to be considered in the formulation of the marinade and process operation. The pH values of meat could easily vary. The pH of marinade is very important to consider as the reaction of the marinade ingredients and the meat proteins is also dependent on pH for the final marinated meat. PSE and DFD meats are meat muscles that are challenging to process since color, texture, and WHC are different from normal meat. The pH and myofibrillar protein structure of PSE and DFD affects the WHC (vanLaak 1999). Processing of PSE and DFD requires alteration of its condition by the marination process. Adjustment of salt concentration and pH of marinade has to be made to effect change in meat pH and electrostatic forces in meat. Marination with salt and polyphosphate had improved PSE condition and matched the normal meat condition (Torley et al. 2000).

Polyphosphates changes the microstructure of the meat by solubilizing salt soluble proteins. Furthermore, polyphosphates help stabilize color and flavor of meat (Farr 1970). In addition, polyphosphates improve tenderness of chicken breast, reduce cooking and frying losses, and improve WHC (Farr 1970; Brotsky 1976; Young and Lyon 1997). The effect of pyrophosphate was more evident than tripolyphosphate on the increase in swelling and decrease in shrinkage of chicken muscle (Shults and Wierbicki 1973). The authors found 0.3% polyphosphate to give raw chicken meat sufficient reduction in cooking loss during cooking of meat. Other functional benefits of polyphosphates, particularly the sodiumtripolyphosphate, were found by Young and Lyon (1997) to decrease red color in cooked meat. Extraction of

proteins from myofibril filaments in the presence of phosphates occurred in different patterns as observed under the phase contrast microscopy. Phosphate increased WHC by extracting the myofibrillar proteins of the meat (Xiong and Kupski 1999c).

Polyphosphate action on myofibrillar protein extraction varies depending on the type of phosphate. Pyrophosphate (PP) and tripolyphosphate (TPP) extracted myosin from both ends of the A band while hexametaphosphate and a control (without phosphate) extracted myosin from the center of the A band. PP was found most effective than TPP and HMP in preventing moisture loss during cooking (Xiong and Kupski 1999c).

Salt reacts synergistically with phosphates in the marinade and enhance WHC (Xiong et al. 1999b). The salt extracts the salt soluble protein in meat. Concentration of salt in marinade has to be carefully considered since high salt concentration can result in a salting out effect and reduces binding properties of meat proteins (Liu et al. 1997). In addition, salt cannot be used to more than 6%, for beyond that it is considered too salty for meat consumers' tastes (Lemos et al. 1999). Workable salt concentration in meat varies. At 2.5% NaCl, the WHC in cooked sausage was at maximum across the pH values with or without phosphate (Puolanne et al. 2001).

Protein solubility of myofibrils increases with increasing salt concentration, however; the protein solubility was markedly affected by phosphate only at low concentrations. At 0.3 M salt concentration, proteins solubility increased about 10 fold. A 0.6 M concentration of salt reduced the effectiveness of phosphates. Xiong and Kupski (1999a) found 8 percent salt to quench the functional properties of phosphate. Phosphate addition significantly increased the WHC in the range of 1.0-1.5% NaCl (Poullane et al. 2001).

Pyrophosphate is a fluidizing agent which can be used to dissociate actomyosin (Wang 1994). Salt and phosphate also affect the denaturation of myoglobin denaturation, and may

influence the intensity of red color in cooked meat (Trout 1989; Lytras et al. 1999). On the contrary, no significant change in shear values was found with the use of salt in the marinade, and in fact, expressible moisture and cooking losses were reduced (Lemos et al. 1999; Froning and Sackett 1985).

Salt decreased pinkness when cooking meat at temperature less than 76^{o}C. On the other hand, sodium tripolyphosphate increased the pinkness in cooked meat products due to the increase in pH (Trout 1989). Addition of salt decreased the stability of myosin and actin. An increase in ionic strength would modify the distribution of surface charges of protein molecules and the intra molecular electrostatic interactions that stabilize the native protein structure. In this case, conformational changes which result from the addition of salt, probably favored the unfolding of proteins thermodynamically.

The effectiveness of the marination process is not only limited to the main functional ingredients, but also the final pH of the meat. The pH plays a significant role in absorption of water in meat. Swelling of myofibrillar protein, which includes both longitudinal and radial changes in fiber was observed when meat pH was increased at both sides of the isoelectric point which in turn increased the WHC of the meat (Karlsson et al. 1996).

Effects of pH was found to increase WHC at increasing levels of pH while the decrease of pH , results in water loss due to the shrinkage of the myofibrillar fibers from the reduced electrostatic charges of the filaments (Poulanne et al. 2001; Hamm 1986). Differences in breast meat color, marinade uptake and cook yield was found in meat samples with extreme variation in muscle pH (Qiao et al. 2002). The same WHC attainment required more salt (2.5%) at pH 5.7 compared to pH 6.3 when only 1.5% NaCl was required (Poulanne et al. 2001).

Marinade Penetration Studies

Xiong and Kupski (1999a) studied the penetration of marinade using different phosphates. The authors found the bigger molecule compound HMP concentration in the outer surface of the meat was higher compared to TPP and PP. TPP and PP diffuse faster because of smaller molecular size, thus concentration was found lower in the outer layer of the meat. In addition, the authors found that the initial 5 min. of the tumbling marination process had the fastest penetration in meat.

Kay (2001) examined the permeability of broiler breasts after marination with salt, phosphate and a combination of salt and phosphate solutions under vacuum tumbling marination. A Confocal Scanning Laser Electron Microscope (CSLM) was used to trace the penetration of the marinade into the meat matrix of the chicken breasts. The result showed the effectiveness of phosphate in increasing the diffusivity of the marinade into the meat significantly when compared to the use of salt alone. Kay (2001) suggested that diffusivity was enhanced by the increase of meat permeability by phosphate. The author used flourescein dye and Lucifer yellow as fluorescing agents. Flourecein and Lucifer yellow have formula weights of 376 and 457, respectively. Both agents are high molecular weight compounds compared to the molecular weight of the marinade ingredients. Most applications of these dyes are for staining living cells and tissues (Horobin 2002a; Horobin 2002b). Issues of limited diffusion of the tracing dye due to molecular size of the fluorescent agents could be a factor in mapping out the real distribution of marinade in meat.

Europium and Yterrbium elements that belong to the lanthanide series of the periodic table maybe a good alternative to flourescein and Lucifer yellow dyes. Europium and Yterrbium have formula weights 151 and 173, respectively. The size of europium and ytterbium are almost

twice smaller than the dyes used by Kay (2001). Europium and ytterbium form luminescent complexes with a variety of organic ligands. Ligands used were thenoylfluoracetone (TTA) and pyridine-2-,6-dicarboxylic acid (Arnaud and Georges 2003). Secondary ligand like trioctylphosphine oxide is added to complete the binding of free ligand binding sites in europium and ytterbium. Fluorescence is emitted upon the excitation of europium and ytterbium ions at 355 or 266 nm. Fluorescence lifetime of europium was reported to be longer and more stable compared to ytterbium (Dimitriev 2003). Europium can complex with organic compounds such as bathophenanthroline disulfonate, a luminescent europium complex that reversibly binds to proteins and nucleic acids (Lim et al. 1997). Europium ion (0.001M) complexed with TTA was found to successfully stain *B. megaterium* cells (Scaff et al. 1969).

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CHAPTER 2

HEAT DENATURATION KINETICS OF THE MEAT PIGMENT, REDUCED MYOGLOBIN AND METMYOGLOBIN[1]

[1]Palang EY, Toledo RT, and Reynolds AE. To be submitted to Journal of Food Science

ABSTRACT

Doneness of cooked meats has been traditionally associated with the absence of the bloody red color in dark meat or of the pink color in white meat. The objective of this study was to investigate the "bloody color defect" due to incomplete denaturation of protein from meat pigment.

Myoglobin pigments were extracted from chicken gizzards and heat treated at various levels of pH, NaCl, and browning agent (BA) concentration. Ground and whole muscle chicken meat (thigh and breast meat fillet) were also evaluated for CIE L*, a* and b* values to validate the result from the model system.

Myoglobin denatured slower than metmyoglobin under the same condition of pH and NaCl concentration. Furthermore, complete denaturation of myoglobin at 1.5%NaCl and pH 6.3 still exhibited red color in the precipitate. A higher CIE a* and lower L* values obtained from ground and whole muscle dark meat confirmed results of the model system. The addition of 0.2% BA and 0.5% salt at pH 6.3 in the meat resulted to lower CIE a* and higher L* values observed from cooked ground and whole muscle thighs than when no BA was used. In "cooked breast meat fillet" samples, pinking was induced after the addition of sodium dithionite.

These results indicate that red discoloration in cooked dark meat can be minimized at pH 6.3 with low salt contents by using a browning agent and by treating the marinade to raise the ORP.

INTRODUCTION

Doneness of cooked meats has been traditionally associated with the absence of the bloody red color in dark meat or of the pink color in white meat. Thus, when consumers encounter pinking or bloody thigh in cooked poultry, the product is rejected because they fear that it could be undercooked. To avoid the bloody color defect, purveyors of precooked meats tend to over cook, adversely affecting tenderness and juiciness of the product.

Overcooked meat loses moisture, resulting in a dry and rubbery texture. In contrast, premature browning in roasted or grilled meat products cooked below recommended endpoint temperatures may give a false sense of doneness. A common consumer practice is to use the disappearance of pink color to indicate doneness even if the final temperature is below the safe endpoint temperature for pathogen inactivation. Metmyoglobin which is formed from deoxymyoglobin and oxymyoglobin may be the dominant pigment in some pieces of meat. Metmyoglobin has a brownish color and retains this brown color regardless of cooking endpoint temperature when heated under favorable conditions. Furthermore, myoglobin derivatives may denature at temperatures below the safe endpoint temperature at low meat pH. When meat pigments are denatured, they become insoluble and precipitate from solution. Thus, when meat pigments are denatured in cooked meat, the liquid which drips out when the meat is cut is almost clear. The absence of a pink color on the meat juice is an indicator of a well done cooked meat. Undercooked meats, especially comminuted meat products, are potential microbiological food safety hazards.

Pinking in cooked white meat for institutional use is a serious problem in industries that supply precooked chicken breast meat and turkey products for delicatessens. Regulatory requirements of a safe final cooking endpoint temperature force the industry to measure product

temperature. However, pinking is still a common occurrence. The unacceptable product is often returned resulting in an economic loss to the processor. This problem needs to be addressed by the industry, although at this time no sure solutions exist. Most poultry products sold pre-cooked are marinated and marination may alter the endogenous properties of the meat. The conditions within the meat could play a role in the elimination of pinking and incidence of premature browning in cooked meats.

Marinade ingredients could potentially cause premature browning and induce stable pink color in meat. Meat properties such as pH and Oxidation-Reduction Potential (ORP) could be altered by the marinades. Furthermore, kinetics of meat pigments denaturation could be affected by the change in pH and ORP. Studies have shown conflicting evidence on the color profile of cooked meat (Lytras et al. 2000; Wallace et al.1982; Cornforth et al. 1986; Torres et al.1988; Trout 1989; Janky and Froning 1973). All observed the same effect of NaCl on denaturation of meat pigments. However, conflicting results were reported on the disappearance of pink color in denatured meat pigments. Trout (1989) in an earlier study, found that at temperature between 52 and 59^{o}C, rate of myoglobin denaturation linearly increased with sodium chloride concentration that made him to conclude that at temperature between 52 and 59^{o}C, sodium chloride decreases the pinkness of cooked meat products. Trout (1989) further found out that pink color in meat cooked at 90^{o}C at pH 6.3 which was attributed to increasing reducing condition in the meat when meat reaches high temperatures. The same author made an inference that no pigment color change will occur at $<76^{o}$C but this color stability is favored when ORP is under reducing condition. Wallace et al. (1982) also found an increase in pink color with increasing NaCl concentration on purified bovine myoglobin. The studies of Torres (1988), Ahn and Maurer (1990) and Cornforth (1986) found that the degree of denaturation of deoxymyoglobin is not a

good representative of the disappearance of pink color in cooked meat. Wallace (1982) and Trout (1989) had attributed disappearance of the pink color to complete denaturation of meat pigments. However, other studies have shown that pink color was still present even in meat cooked to an endpoint temperature much higher than denaturation temperature of the meat pigments.

Several factors have been found to contribute to the development of a pink cooked meat color. Contamination from nitrate/nitrite and carbon monoxide has been identified as one cause (Geilesky et al. 1998; Holownia et al. 2004). However, even in the absence of ligands (nitrate/nitrite and carbon monoxide) pink color in cooked meat could possibly be still present even at the complete denaturation of meat pigments. Meat pH and ionic strength of fluids within the meat were initially identified as one of the major factors that affect the stability of the pink color in meat. Commercially available browning agents such as Maillose (Red Arrow) and CharSol (Red Arrow) were formulated to help remove pink color in cooked meat.

The objective of this study was to determine the effect of pH, NaCl, and browning agent concentration on the prevention of the development of a stable pink color in tumble marinated meat. The denaturation of myoglobin and metmyoglobin was studied in a model system. Ground chicken leg quarters, whole muscle leg quarters, and chicken breast were used to validate results of the model system studies. In addition, we hypothesized that a pink color in cooked white meat can be induced through alteration of the ORP after cooking.

MATERIALS AND METHOD

Materials

Chicken gizzards used for extracting the meat pigments were obtained from a local grocery store.

All chemicals were all analytical reagent grade obtained from J.T. Bakers Inc. (Phillipsbers, USA). Reagents used were ammonium sulfate, ammonium hydroxide, ferric sulfate, sodium bisulfite, potassium ferricyanide, sodium chloride, monosodium phosphate, disodium phosphate, EDTA, sodium dithionite.

Browning accelerator (BA) was a caramel like preparation obtained from Red Arrow International, Manitowoc, WI.

Myoglobin Derivatives Isolation

Chicken gizzards purchased from a local grocery store were used to extract myoglobin and derivatives. Gizzards were used because of the high myoglobin content. The extraction of myoglobin derivatives and conversion to deoxymyoglobin and metmyoglobin followed the procedures of Matsuoka et al. (1987) and Janky and Froning (1973), but were modified for this experiment. 500 g chicken gizzards were passed twice through a Hobart grinder. After grinding, the ground gizzards were mixed with 1.5 volume of 0.5 mM EDTA aqueous solution and followed with the pH adjustment to 8.0 using 1.0 M of ammonium hydroxide. The slurry formed and was set aside and kept for 1 hr. in a walk-in cooler prior to centrifugation.

The insoluble materials from the gizzard slurry were removed by centrifugation (Sorvall Refrigerated-Auto) at 2000 x G for 15min. at 4^{o}C. After centrifugation, the supernatant was decanted and pooled in a container for fractionation while the precipitate was discarded. The pooled supernatant (pigment extract) was then treated with 75% (0.525g/ml extract) saturated ammonium sulfate and the pH was adjusted to 7.8 using 1.0 M ammonium hydroxide to precipitate the hemoglobin (O'Brien et al. 1992). Insoluble materials formed after pH adjustment was separated from the clear solution by centrifugation. The supernatant should have yielded

51% myoglobin and 0.092% hemoglobin in solution (O'Brien et al. 1992). The supernatant was collected and dialyzed to obtain purified myoglobin.

Myoglobin Derivatives Purification

Salts were removed from the pigment extract by dialysis through a membrane tube with MW range cut off of 12-14,000. Both ends of the dialysis tubes were closed prior to placement in an aluminum tub filled with deionizer water in a walk-in refrigerated room at 2^{o}C. The deionizer water was constantly stirred by a magnetic stir bar driven by a magnetic stirrer under the tub. Deionized water was emptied from the tub and replaced with fresh water at 4 h intervals. All components of the extract with MW< 12,000 were removed through the dialysis tubes. The dialysis procedure was continued until molarity of pigment extract was reduced to < 0.0001 M as tested by an osmometer (Osmette A, Precision Systems Inc). Ionic species in the pigment extract must be present in negligible amount. After dialysis, the purified myoglobin derivatives were twice filtered first in Whatman #1 Qualitative filter and then through Cellulose Nitrate Membrane filter with 0.45 µm porosity under 15 inches vacuum.

The filtered myoglobin derivatives were then concentrated in a rotary evaporator (Rotavapor, Brinkman) with the water bath temperature set at 27^{o}C, and an absolute pressure of 160 mBar. The pigment extract was concentrated to half of its original volume. The concentrate was analyzed for myoglobin content and the concentration was adjusted by dilution with deionized water. The concentrated myoglobin derivatives was then stored in a walk-in refrigerated room maintained at 2^{o}C and used as a stock solution for all experiments.

Kinetics Study of Myoglobin and Metmyoglobin Denaturation

A 100 mL aliquot of the stock solution of myoglobin derivatives was removed and treated with 40 mg sodium bisulfite to convert the myoglobin derivatives to its reduced form, deoxymyoglobin. Another 100 mL aliquot was treated with 75mg of potassium ferricyanide to produce a metmyoglobin solution. Complete conversion of myoglobin to deoxymyoglobin and metmyoglobin was checked by taking the ratio of absorbance at 507nm/573nm for deoxymyoglobin and 473nm/597nm for metmyoglobin and the amounts were obtained from a curve of absorbance ratio vs. pigment concentration given by Broumand et al. (1958).

An 8 mL of either myoglobin or metmyoglobin extract was pipetted into a 13 x 100 mm test tubes. The sodium chloride was then added to the test tube to obtain the desired ionic strength. pH was adjusted using either monosodium or disodium phosphate. BA (Red Arrow, Manitowoc, WI), a browning agent, was then added into the test tube at 0, 5 and 10% concentration to see its effect in the denaturation of pigment protein. The pH of the solutions was adjusted to 5.5, 5.9 and 6.3, while the NaCl concentrations were 0.8, 1.15 and 1.5% in the solution. Each of the test tubes was then flushed with nitrogen gas and sealed with a rubber stopper to maintain anaerobic condition while heating in a water bath. Temperature of the water bath was adjusted near 2 degrees Celsius higher than the endpoint temperatures of the samples. The temperatures sample endpoint temperatures used for the experiment were 65, 75 and 85^{o}C and times of heating were 0, 5, 15 and 25 min. The time of heating was started once the temperature of the solution inside the tube reached the desired temperature. Treated test tubes were immediately transferred into a water-ice slush to cool it down and arrest the denaturation reaction.

Denatured pigments are insoluble and will precipitate from solution. After cooling, contents of the treated tested tubes were transferred to centrifuge tubes and centrifuged at 1380 x g (Centrific Model 228, Fisher Scientific) at ambient for 15 min. The supernatant was decanted and analyzed for total heme pigment by converting the myoglobin derivatives into cyanometmyoglobin. The supernatant from each treated tube was mixed with 100 µL of a solution containing 60mM $K_3Fe(III)(CN)_6$ and 80 mM NaCN to convert the pigments to cyanometmyoglobin. The cyanometmyoglobin was quantified by spectrometer (Spectronic Genesys) as described by Rickansrud and Henrickson, 1967 and Drabkin, 1950:

Total Heme(moles/Liter) = $(A_{540}-A_{700})$ x D/E x d

Where:

D = dilution factor

d = light path

E = molar extinction coefficient at 540; 11,300 (L/cm mole)

An optimization experimental design was set-up using Optex procedure of SAS software. The absorbance data was statistically analyzed and a fitted into a model using Stepwise Regression procedure of SAS software.

Chicken Sausage Preparation

Chicken leg quarters were purchased from a local grocery store. On the same day, the chicken leg quarters were deboned manually. Visible fat and connective tissue was removed from the meat. The deboned meat was ground twice through a meat grinder attachment to a table top kitchen blender (KitchenAid Professional, St. Joseph, MI). The ground meat was

proportioned and mixed with different marinades. Each treatment was a combination of the different concentration of NaCl (0.5 and 1.5%), BA (0, 0.2%), pH (5.5 and 6.3) and the rest was deionized water. Citric acid was added to the ground meat until the ground meat mixture desired pH was attained. The amount of marinade added to the ground meat was 10% of the ground meat original weight. Meat was mixed with the marinade manually. The meat pH was measured using a meat piercing pH electrode (Flexiphet). The ground meat and the marinade was combined and mixed thoroughly. Mixing was stopped when pH measurements on the ground meat was uniform throughout the batch.

The ground meat slurry was stuffed into 2.54 cm diameter cellulose casings using a stuffing horn attachment to a meat grinder attached to the KitchenAid mixer. Stuffed sausages were linked by manually tying with a string to an average length of 2 cm. The links were stored in the freezer at – 18^{o}C until used. Prior to use, they were thawed overnight at 4^{o}C.

Split-splot experimental design was the design of the experiment. The temperature was randomized and within the temperature the time of heating of the sausage sample was randomized. The experiment was replicated twice. Sausage links were heated in a water bath set at 60, 70, and 80^{o}C and sausages were heated 10, 20, and 30 min. one at a time. Heating time started once the internal temperature of the sausage reached the temperature of the water bath. A Type T thermocouple was inserted into the center of the sausage. After heating the sausage link was immediately submerged into an ice-water slush to arrest temperature induced changes.

Treated sausage links at ambient temperature were cut longitudinally along the diameter and the cut surfaces were exposed to air for 1 min under ambient conditions before measuring the CIE L*, a* and b* values using a reflectance spectrophotometer with 10^{o} view angle, D65

illuminant and small area/spec inclusion (ACS CS-5 Chroma-sensor, Applied color system, Inc). Each treatment was replicated twice.

Thigh Meat Fillet Sample Preparation

Chicken leg quarters purchased from a local grocery store were used for the experiment. Skin was removed and meat was manually separated from bone. Deboned meats were stored at 2^{o}C in a walk-in cooler overnight prior to the experiments. Eight marinades were prepared from the combination of different levels of NaCl (0.5 and 1.5%), pH (6.3 and 5.5) and BA (0, 0.2%) concentration. Marinade was added to the meat in the vacuum tumbler at 10% of meat weight.

Marination was conducted for 25min. at 133mBar absolute pressure, 10 RPM and 4^{o}C. After tumbling, the meat was stored overnight at 4^{o}C in a walk-in cooler before cooking.

Cooking was done in an electric clam shell grill with top and bottom heating plates set at the target endpoint temperature. End point cooking temperatures in the meat were 65, 70, 75 and 85^{o}C. A type T thermocouple was inserted into the geometric center of the deboned chicken meat to monitor its internal temperature. The meat was immediately removed after reaching the target temperature and immersed in water-ice slush. Each treatment was replicated twice and the design of the experiment was a split-splot experimental design.

The cooled meat was cut laterally at the half-thickness and exposed to air for at least 1 min. under ambient condition before measuring the CIE L*, a* and b* values. A Minolta (Model 410) spectrometer with 2^{o} view angle and C illuminant was used to measure the CIE L*, a* and b* values.

Pinking Cooked Chicken Breast

Boneless chicken breasts were purchased from a local poultry processing plant. Excess fats were trimmed out manually. After trimming, the meat was marinated by vacuum tumbling at 133 mBar absolute and 10 RPM at different time and temperature combinations. The treatments were the following: a) 12°C/25min., b) 12°C/10min. + 4°C/15min. c) 8°C/10min. + 4°C/15min., d) 8°C/25min., and e) 4°C/25min. The marinade consisted of NaCl (1%) and sodiumtripolyphoshate (STP) (0.35%). The rest of the marinade was deionized water, and marinade absorption was set to 20% of the meat original weight. Citric acid (0.0027%) was used to adjust the marinade pH to 5.9.

The marinated chicken meat was cooked in an oven set at 177°C (Lyon and Lyon 2002) to an internal endpoint temperature of 71.1°C. Temperature was monitored by a type T thermocouple inserted in the geometric center. Cooked meat was allowed to cool at ambient temperature, packed inside a resealable polyethylene bag and stored at 4°C in a walk-in cooler until analyzed after 24 h. Relative Oxidation-Reduction Potential (ORP), CIE L*, a* and b*, and pH were measured on raw breast chicken meat before and after marination. Furthermore, the relative ORP, pH and CIE L*, a* and b* values were measured after cooking, and after 1, 3 and 5 day of storage. After day 5, meat was excised though the half-thickness and sodium dithionite, a reducing agent was sprinkled on the exposed surface. The relative ORP and CIE L*, a* and b* values were measured. The relative ORP value was standardized against an ORP Standard (Thermo Orion Application Solution) that read +420 mV at 25°C. The ORP surface probe (Cole Parmer) was rinsed with deionized water before each measurement on the surface of the chicken breast sample. The ORP reading was taken after 1 min. Before taking the next measurement, the ORP prove was submerged back to the deionized water and ensure that the ORP reading has a

similar reading initially. A Minolta reflectance spectrophotometer with 2 ^{o} view angle and C illuminant was used to measure the CIE L*, a*, and b* values.

RESULTS

Model System Studies

Sodium dithionite, a reducing agent, converts myoglobin to deoxymyoglobin. Ferric cyanide on the other hand is an oxidizing agent and converts myoglobin to metmyoglobin. The percentage conversion of myoglobin to deoxymyoglobin and metmyoglobin was verified by taking the ratio of absorbance at wavelengths 507nm/573nm (deoxymyoglobin) and 473nm/597nm (metmyoglobin) and ratios were converted to percentages from a curve reported by Broumand et al. (1958). The minimum levels of deoxymyoglobin or metmyoglobin in the pigment extracts used in the model system studies were at least 90%. The absorbance ratio 507 nm/573 nm was at least 0.8 and the absorbance ratio 474nm/507 nm was at least 3.0 based from the absorbance ratio curve by Broumand et al. (1958) to obtain a minimum 90% deoxymyoglobin and metmyoglobin concentration, respectively. The rest of the myoglobin was present as oxymyoglobin.

The absorbance values of heated pigment extract supernatants after conversion to cyanometmyoglobin by the addition of 100 µL of a solution containing 60mM $K_3Fe(III)(CN)_6$ and 80 mM NaCN were statistically analyzed using stepwise regression to find the best fit mathematical model for deoxymyo globin (Table 2.1) and metmyoglobin (Table 2.2). The model was used to generate the response surface model graph of both the denaturation of deoxymyoglobin and metmyoglobin extracts. The main effects (pH, BA, time, NaCl and temperature) were found to be statistically significant. Main interactions effects between temperature x pH, temperature x BA, pH x BA, and NaCl x BA were found to significantly

affect the denaturation of both deoxymyoglobin and metmyoglobin. Both deoxymyoglobin and metmyoglobin showed the same significant main effects and main interaction effects in each corresponding models. The model followed first order kinetics that agreed with the work of Geilesky et al. (1998) also in aqueous myoglobin extracts. The model was found not a good predictor of deoxymyoglobin and metmyoglobin protein denaturation when pH and BA levels of the samples were at 6.3 and 0 percent, respectively (Figs. 2.1 and 2.4). The denaturation of pigment protein was higher at 0.8 percent instead at 1.5 percent in the absence of BA compound.

Denaturation of myoglobin was not complete at 60^{o}C anytime between 0 to 30min. of water bath heating (Fig. 2.1). Degree of denaturation increased as the deoxymyoglobin was exposed to increasing temperature. Amount of pigment protein denatured was higher at 80^{o}C (Fig. 2.3) than at 70^{o}C (Fig. 2.2) at any combination of pH, NaCl and BA concentration. The response surface model graphs among the three temperatures showed an increasing amount of protein denatured when NaCl concentration was increased. Furthermore, denaturation was found highest at 1.5 percent NaCl in the absence of BA. The addition of BA had a reverse influence to the denaturation of protein with salt concentration.

The exposure of metmyoglobin to heat showed similar behavior as the myoglobin. At 60^{o}C, no complete denaturation was observed (Fig. 2.6) at any given time of heating from 0 to 30 min. Amount of denatured pigment protein in metmyoglobin was highest at 80^{o}C (Fig. 2.6) and followed next at 70^{o}C (Fig. 2.5). The effect of levels of salt, pH, and browning agent were found to follow the same trend as the deoxymyoglobin. The RSM model for both deoxymyoglobin and metmyoglobin samples showed that deoxymyoglobin was more heat resistant than metmyoglobin. Protein of the deoxymyoglobin pigment was more stable from denaturation than the metmyoglobin under any condition of pH, BA, and NaCl. The results that we found on this

experiment, collaborating findings by Hunt et al. (1999) on relative stability of the two pigments to heat denaturation.

Responses of deoxymyoglobin and metmyoglobin to denaturation as affected by NaCl, pH, and BA followed similar trends. The extent of pigment denaturation at a specific temperature increased with increasing NaCl and BA concentration, and with decreasing pH. The significant NaCl x BA interaction is manifested by higher denaturation at lower NaCl concentration in the presence of BA compared to the higher NaCl concentrations. We hypothesized that complete denaturation of myoglobin and its derivatives will result in the disappearance of the pink color in meat. Experimental results invalidated this hypothesis. Although the color of the supernatant and precipitate of the heated pigment extract were not measured instrumentally due to the assumption that lesser pink color will be observed in extracts with more denatured pigment, visual observation of these colors showed differences in the shade of color. The color difference between supernatant and precipitate was visually obvious both for myoglobin (Fig. 2.7) and metmyoglobin (Fig. 2.8). Either a pinkish or reddish color was observed on both supernatant and precipitate of the heated pigment extract at higher NaCl levels. At lower NaCl levels, the red or pink color was faint or was absent. Heated extracts with BA appeared to have reduced pink color intensity compared to their counterparts with the same treatment without BA. BA had a very light clear brownish color which could possibly mask the pink color. Visual observations could not distinctly establish loss of the pink color and meat pigment denaturation.

The extent of pigment denaturation was not directly related to the disappearance of pink or red color in the solution at high NaCl levels. The disappearance of pink or red color from both supernatant and precipitate was affected by the NaCl level BA concentration. Pigment that was 100 percent denatured was observed to still exhibit the pink color on treatment especially when

1.5 percent NaCl concentration in the extract (Fig. 2.7 and Fig. 2.9). Although two solutions may exhibit the same extent of pigment denaturation, one may have a pink color while others may have a grayish-brown color. Furthermore, the rate of pigment denaturation was pH dependent, the higher the pH the slower the rate at the same temperature. Higher heating temperatures accelerated the rate.

Color Changes in Heated Chicken Sausage and Leg Quarters

While making the chicken sausage, color difference could already be seen in the different formulations (Figs. 2.9 – 2.17). Uncooked chicken sausage links with higher NaCl levels (1.5%), higher pH (6.3) and without BA had lower L* but higher a* values compared to the lower salt (0.5%) and pH (5.5) formulations. Sausage links exhibiting lower L* and higher a* values showed more intense pink or red color while the higher L* and lower a* values had a pale redish-brown color. However, formulations with 0.2% BA, exhibited higher a* values at 0.5% NaCl than at 1.5% (Figs. 2.10, 2.11, 2.15, and 2.16), the opposite effect of NaCl levels in the absence of BA.

Sausages cooked at different heating medium temperatures exhibited different colors across all treatments. Heating at 70^{o} and 80^{o}C resulted in a significantly higher change in L* and a* values from raw samples compared to 60^{o}C. A significant correlation was found between the heating medium temperature and the L* ($r=0.542$, $P<0.05$) and a* ($r=-0.684$, $P<0.05$) values. Prolonged heating of the sausage links increased L* value and decreased a* value. The heating time required to eliminate pink or red color was shortened with increasing temperatures. At lower temperature (60^{o}C) the pink color was still present after 30 min. of heating in samples with pH 6.3 both at 0.5 and 1.5 percent salt with or without 0.2 percent BA (Fig. 2.13-2.16). In samples with pH 5.5, pink color was only observed in samples added with 0.2 percent BA and

1.5 percent NaCl (Fig. 2.12). The color of heated sausage links after 30 min. at 60^{o}C was the same as in the unheated samples but at 70^{o}C or higher, the color changed significantly from the raw sample. Increased pH significantly increased a* values ($r=0.369$, $P<0.05$) of the heated samples. Differently formulated samples exhibited different color shades and intensity at the same endpoint temperature as demonstrated by the CIE L*, a* and b* values among samples. The samples that were formulated with higher concentration of salt and with higher pH without BA had higher a* value and lower L* value that appeared more red (Fig. 2.9 and 2.13). The addition of 0.2 percent BA into all the samples, decreased the a* values of all samples except in samples with 0.5 percent NaCl (Fig. 12 and 16).

The deboned leg quarter samples with 0.2% BA had lower a* values as than those with no BA that was similarly observed from the sausage samples. Deboned leg quarter fillet samples heated to 65^{o}C still exhibited red color (Fig. 2.17). However, results showed that samples with 1.5 percent NaCl and pH 6.3 had higher a* values. Fig. 20 shows an increase in L* and decrease a* values of the samples heated to 70^{o}C compared to samples heated to 60^{o}C. At this endpoint heating temperature of the deboned leg quarter samples, red color was still obviously observed in all samples. A continuing trend was observed in the sample L* and a* values heated at 75^{o}C. L* value generally increased and a* value decreased but still red color was apparent (Fig. 19). However, heating the samples to 85^{o}C, red color intensity was diminished and visually undetectable (Fig. 20). The disappearance of pink or red color on heating was faster at lower pH and lower NaCl levels without 0.2% BA. However, when 0.2% BA was added, higher NaCl levels was found to have a* values. The change in CIE L* and a* values in sausage and chicken leg quarter fillet to different levels of pH, NaCl and BA concentration was the same. Furthermore, collaborated the color observed from the myoglobin model system studies.

Pink Color Development in Normally Colored Cooked Meat

Boneless chicken breasts tumble marinated to a target meat pH of 5.9 and 1.0% NaCl was in the middle range of pH and NaCl levels used in earlier experiments (Table 2.8). This pH and NaCl levels would favor meat pigment degradation and minimize cooking loss. CIE L*, a* and b* values of the heated meat changed significantly compared to raw meat. The exposed faces of the excised cooked sample exhibited a "cooked chicken meat appearance" in all samples on visual evaluation. Thus 1.0% NaCl and 5.9 pH in the raw marinated meat did not result in a "pinking" problem in the cooked meat. The pH of the marinated meat increased after cooking that was similarly observed by Geilesky et al (1998). Final pH of cooked meat may affect the disappearance or appearance of pink color during storage.

The CIE L*, a* and b* values of the stored cooked breast meat did not significantly change from day 0 to day 5. The L*, a* and b* values were higher on the meat surface that was on top during heating compared to the exposed excised middle and bottom surfaces (Tables 2.3-2.7). Higher a* color values on the top surface could be attributed to moisture evaporation which dried the surface and accelerated the Maillard reaction. Drying of the bottom surface which was in contact with the cooking pan was not as much as the top exposed to air. Up to day 5, there was still no appearance of the pink color in the stored cooked meat. The pH values in cooked meat remained constant while ORP values increased slightly (Table 2.8). The ORP value increased towards the positive direction indicating a change in to meat to an oxidizing condition. After observing no significant change in the CIE L*, a* and b* values, the surfaces were treated with sodium dithionite powder to change the ORP towards reducing conditions. The shift in ORP with sodium dithionite treatment was as much as 200 mV. This treatment increased a* value rapidly regardless of marination schedule process. Table 2.5 showed an increase in L* and a* values

after sprinkling sodium dithionite on the surface of the chicken breast fillet tumble marinated to 8^{o}C/10min. + 4^{o}C/15min. Breast fillet tumble marinated to 8^{o}C/25min. (Table 2.6) and to 4^{o}C/25min (Table 2.7) showed increase in L* and a* values as well. The increase of a* values was inversely proportional to the ORP value of the meat (Table 2.9). The a* value of the cooked breast meat fillet increased after adding sodium dithionite. Furthermore, b* values also decreased. Photographs of cooked meat fillets before and after the addition of sodium dithionite can be seen in Fig.2.21.Threshold CIE L*, a* and b* values of visible pink color appearance was not determined in this experiment. However, the change in CIE L*, a* and b* values of the interior surface of the cooked meat was measured. The interior surface of the chicken was the surface of interest since meat consumers evaluate meat "doneness" in meat interior surface. Obvious pink color was observed in the interior surface of the meat samples with at least 6.0. Furthermore, b* values of cooked meat fillets decreased by about 2 units which may contribute to the increase of the visual red color intensity of the samples.

Holownia et al. (2003) determined the threshold pink L* and a* values in chicken breasts. Based on Holownia threshold a* value of 3.8, a 6.0 a* value must show a bright pink color. The discrepancy in a* values between these studies could be attributed to the different spectrometer equipments used on both experiments. Different view angles and illuminant sources in both equipments could make a difference in CIE values. In addition, the comparison of ORP values in this experiment to other experiments should be carefully evaluated. Each ORP probe has its own off-set ORP value. In order for the ORP values reported in this experiment to be replicated or compared to other ORP measurements, the ORP probe off-set value should be corrected using an ORP standard (Thermo Orion Application Solution) used in this experiment that read +420 mV at 25^{o}C.

DISCUSSION

The increased denaturation of meat pigments at high NaCl levels was observed by Ahn and Maurer (1989), Lytras et al. (2000), Wallace et al. (1982), Cornforth et al. (1986), Torres et al (1988), Trout (1989), Janky and Froning (1973). Hemoglobin was reported to dissociate more at higher salt concentration (Antonini and Brunori 1971). The dissociation of hemoglobin or myoglobin could open the globular protein exposing more of the molecule making it more vulnerable to the effects of pH and heat. In addition, pH in acidic range favored the dissociation of the heme moiety in the myoglobin. On the other hand, high pH increases the affinity between the heme moiety and the globular protein making the latter more stable to heat denaturation (Antonini and Brunori 1971). These studies appear to support our observations on myoglobin denaturation as affected by NaCl and pH.

The presence of BA was also a significant main effect variable in the myoglobin denaturation mode (Table 2.1 and 2.22). BA, a caramel-like smoke fraction that contains mostly acids and carbonyl compounds also stabilized the globular protein at the higher NaCl levels. BA was developed commercially to be a Maillard browning accelerator. Most likely, it reacts with proteins to expose terminal amino groups that can react with the carbonyls in BA. This accelerates pigment degradation at low NaCl levels. However, at high NaCl levels, ionic bond in the protein molecule slows down protein-protein interaction resulting in a slower denaturation rate even in the presence of BA.

Color change from red or pink to gray or grayish brown after heating was not directly related to the extent of pigment denaturation. Degree of myoglobin and deoxymyoglobin denaturation may be used as a predictor or indicator of cooked meat doneness but only under certain conditions. The supernatant and precipitate of heated pigment extract at high NaCl levels

exhibited pink color even when the pigments were totally denatured (Fig. 2.7 and 2.8). Similarly, metmyoglobin can be reduced deoxymyoglobin or to a reduced form of hemochrome (Tsukahara and Ishida 1991). However, metmyoglobin will appear brown regardless of endpoint cooking temperature when oxidizing conditions exist in the meat (Hunt et al 1999). Trout (1990) found that the rate of metmyoglobin formation was increased as NaCl concentration increased in ground beef, pork and turkey meat during refrigerated storage. On the other hand, studies conducted by Torres et al. (1988) showed no significant change to the rate of metmyoglobin formation at concentration as high as 4% salt (NaCl). Trout (1989) in an earlier study, found that at temperature between 52 and 59^{o}C, rate of myoglobin denaturation linearly increased with sodium chloride concentration that made him to conclude that at temperature between 52 and 59^{o}C, sodium chloride decreases the pinkness of cooked meat products. Trout (1989) further found out that pink color in meat cooked at 90^{o}C at pH 6.3 which was attributed to increasing reducing condition in the meat when meat reaches high temperatures. Trout (1989) made an inference that no pigment color change will occur at <76^{o}C but this color stability is favored when ORP is under reducing condition. Wallace et al. (1982) also found an increase in pink color with increasing NaCl concentration on purified bovine myoglobin. Our results concur with the studies of Torres (1988), Ahn and Maurer (1990) and Cornforth (1986) that the degree of denaturation of deoxymyoglobin is not a good representative of the disappearance of pink color in cooked meat. Rather, the ORP value of the meat influences the color of meat pigments whether denatured or native. Wallace et al. (1982) and Trout (1990; 1989) may not be aware of the ORP value of the samples used in their experiments as affected by increasing NaCl concentration or the presence of other additives.

Denatured protein also known as hemochrome, assumes the brown color when Fe^{3+} is in the heme. Otherwise, a hemochrome with Fe^{2+} will exhibit a pink color (Osborn et al. 2003). Fe^{2+}, in the reduced form of the heme moiety in myoglobin allows strong affinity to ligands at its sixth ligand binding site to produce bright red or pink color. On the other hand, Ferric or Fe^{3+} imparts brown color by attracting O_2 to its sixth binding site. Exposure of the heme compound to favorable conditions will have a bearing on the oxidation state of the iron. The valence state of the iron is primarily affected by the oxidation-reduction potential in the meat. Under a high oxidation reduction potential (positive ORP value), oxidation is favored while reduction is favored at decreased ORP value. Lien et al. (2002a), Ghorphade and Cornforth (1993), Maga (1994), Lytras et al. (1999) and Geilesky et al. (1998) inferred that the degree of globular protein denaturation was correlated to the disappearance of pink color in meat. The latter linked the pink color appearance in cooked meat from contamination with nitrate/nitrite, carbon monoxide and exposure to high pH which is a proven fact but did not mention the possibility of reduction of hemochrome and its affinity to oxygen ligand..

ORP value of meat should be carefully considered in the formulation of marinade or by the condition in and around the meat. In the case of marinade, ingredients such as the salt, phosphate and pH may directly affect meat ORP. Each ingredient can either increase or decrease the ORP value of meat. We showed levels of marinade ingredients that influences strongly ORP value of meat. Citric acid, NaCl and BA decreased ORP value (towards the reducing region) with temperature increase. On the other hand, the phosphate ingredient increased the ORP value. In addition, salt and phosphate are ionic compounds that contribute to the ionic strength in marinated meat. The use of ionic strength as a predictor of pink color disappearance in cooked meat is not reliable because it is not just the ionic strength but the influence of these components

on the ORP that determines whether conditions are right or not for the cooked meat to exhibit the pink color.

According to Nerns't equation ORP is directly proportional to increase in temperature. At room temperature, the ORP value of the marinade was measured initially in the range 490 to 510 mV while values decreased to range 50 to 100 mV when the marinade solution was heated to 60^{o}C. Nam et al. (2002a) studied the effect of irradiation on turkey meat and found lowered ORP on the meat surface to reducing conditions where heme pigments can bind to ligands to increase intensity of pink color. Ahn and Maurer (1990a) reported that denatured pigments can bind to different ligands and develop pink color, and thus even totally denatured meat pigments can still posses pink color. Nam et al. (2002b) also postulated that reducing conditions led to Fe^{3+} to Fe^{2+} transformation creating high affinity for ligands that produced red color in irradiated turkey breasts. We showed in our studies that addition of sodium dithionite to obtain reducing conditions in cooked chicken breast meat resulted in the increase of a* value (Tables 2.5, 2.6 and 2.7). Cooking is obviously performed at high temperature which can significantly change the ORP value as meat is cooked. However, on cooling, ORP value again decreased as shown by just a small difference between raw and cooked meat OPR while pH value increased on cooking. This showed that ORP values of a solution could change differently with temperature change and presence of additives compared to meat tissue. ORP and pH values in chicken breast meat have opposing effects on pink color intensity in cooked meat. ORP values at reduced levels can be compensated by low pH to prevent pinking and vice versa. The final meat color of cooked meat is a result of complex mechanisms. Myoglobin may exist in different forms each form exhibits a different dynamic on color changes in cooked meat. For example, the disappearance of pink color at high heat temperature could be due to the denaturation of meat pigments and reduces

affinity of oxygen to the meat pigment at higher temperature (Antonini and Brunori 1971; Lien et al. 2002b). Lower temperature (60 –70^{o}C) denatures protein but the link between the heme iron and globin is still intact while at higher temperatures (85 to 100^{o}C) linkage between the globin and the heme moiety may be separated (Han et al. 1993). Masking of pink color could be possible from the products of Maillard-type reactions between sugars and amino groups and caramelization of carbohydrates (Renere 1990).

On the other hand, the appearance of pink color in cooked meat could be due to the reducing conditions in the meat. The denaturation of globular protein under reducing condition, allows the amino acid side chains in globin to draw away from the heme moiety. As a result, the heme moiety is exposed and makes it open for reacting with ligands found in the amino acid side chain of the globin. In particular, histidine, an amino acid side chain, would immediately bind with oxygen by electrostatic interaction to the sixth free ligand binding site of the heme. As a result, the binding of histidine increases the affinity of the oxygen 1000 times Olson et al. (1997). Oxygen attachment to the sixth ligand binding site when heme is in the form of Fe^{2+} will make the pigment exhibit the red color.

CONCLUSION

Complete denaturation of myoglobin derivatives in meat was not an indicator of meat "doneness" judged by the disappearance of the bloody red color. Higher pH and NaCl concentration increased the incidence of pink color in cooked meat. Furthermore, completely denatured myoglobin could appear as red or brown depending on the oxidation reduction potential in the meat. Ionic strength was not a good predictor of whether or not pinking will occur since specific ionic species may either increase or decrease ORP value. In addition, hemochrome can be reduced when ORP value is in the reducing range (Hunt et al. 1999;

Cornforth et al. 1986). ORP value of meat plays a significant role to the oxidation state of Fe in the heme whether the meat is raw or cooked and therefore determines the color of the meat pigments. Furthermore, BA, a browning agent contributed to the decrease in pink color intensity in cooked meat by either masking the pink color through Maillard reaction browning or by reacting with the globin to prevent the formation colored ligands.

DEOXYOGLOBIN (R^2 = 0.758)		
Parameter	**Estimate**	**Pr > T**
INTERCEPT	-777.5	0.0006
TP	14.25	0.0001
PH	104.7	0.0060
SLT	26.42	0.0006
MAIL	30.10	0.0001
TI	0.3176	0.0115
TP*PH	-2.028	0.0002
TP*MAIL	-0.1278	0.0025
PH*MAIL	-2.448	0.0190
SLT*MAIL	-5.006	0.0001

Table. 2.1. RSM model of deoxymyoglobin as a function of temperature, pH, BA and NaCl concentration. TP = temperature, ^{o}C; MAIL = browning agent; SLT = NaCl; and TI = time,min.

METMYOGLOBIN (R^2 = 0.674)		
Parameter	**Estimate**	**Pr > T**
INTERCEPT	- 615.7	0.0180
TP	11.06	0.0026
PH	78.95	0.0712
SLT	32.16	0.0003
MAIL	35.80	0.0001
TI	0.3257	0.0252
TP*PH	-1.516	0.0138
TP*MAIL	-0.1126	0.0204
PH*MAIL	-3.555	0.0036
SLT*MAIL	-5.189	0.0002

Table. 2.2. RSM model of metmyoglobin as a function of temperature, pH, BA and NaCl concentration. TP = temperature, ^{o}C; MAIL = browning agent; SLT = NaCl; and TI = time,min.

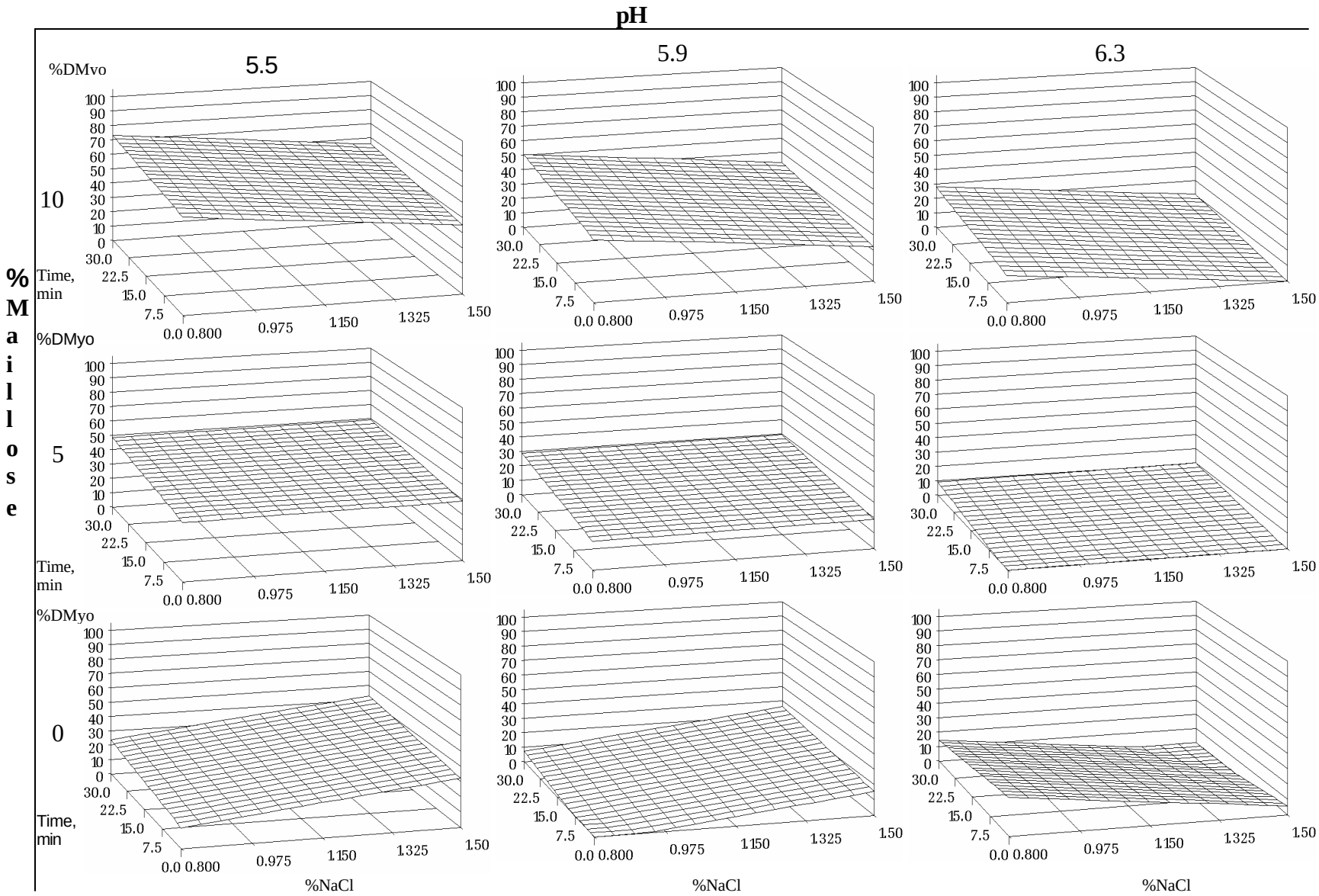

Figure 2.1. Response Surface of deoxymyoglobin denaturation at 60°C from model equation of deoxymyoglobin in Table 2.1.

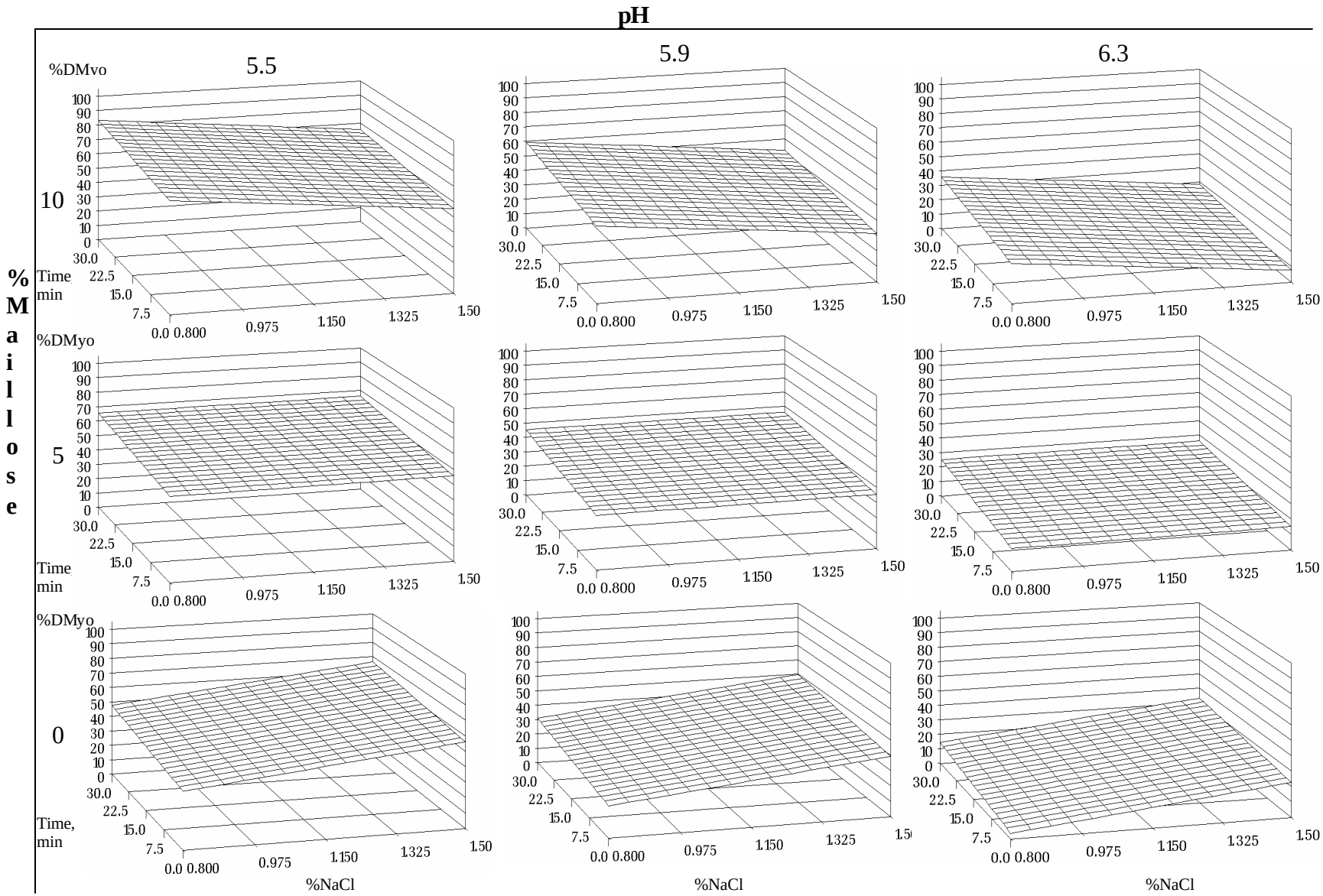

Figure 2. 2. Response Surface of deoxymyoglobin denaturation at 70^{o}C from model equation of deoxymyoglobin in Table 2.1.

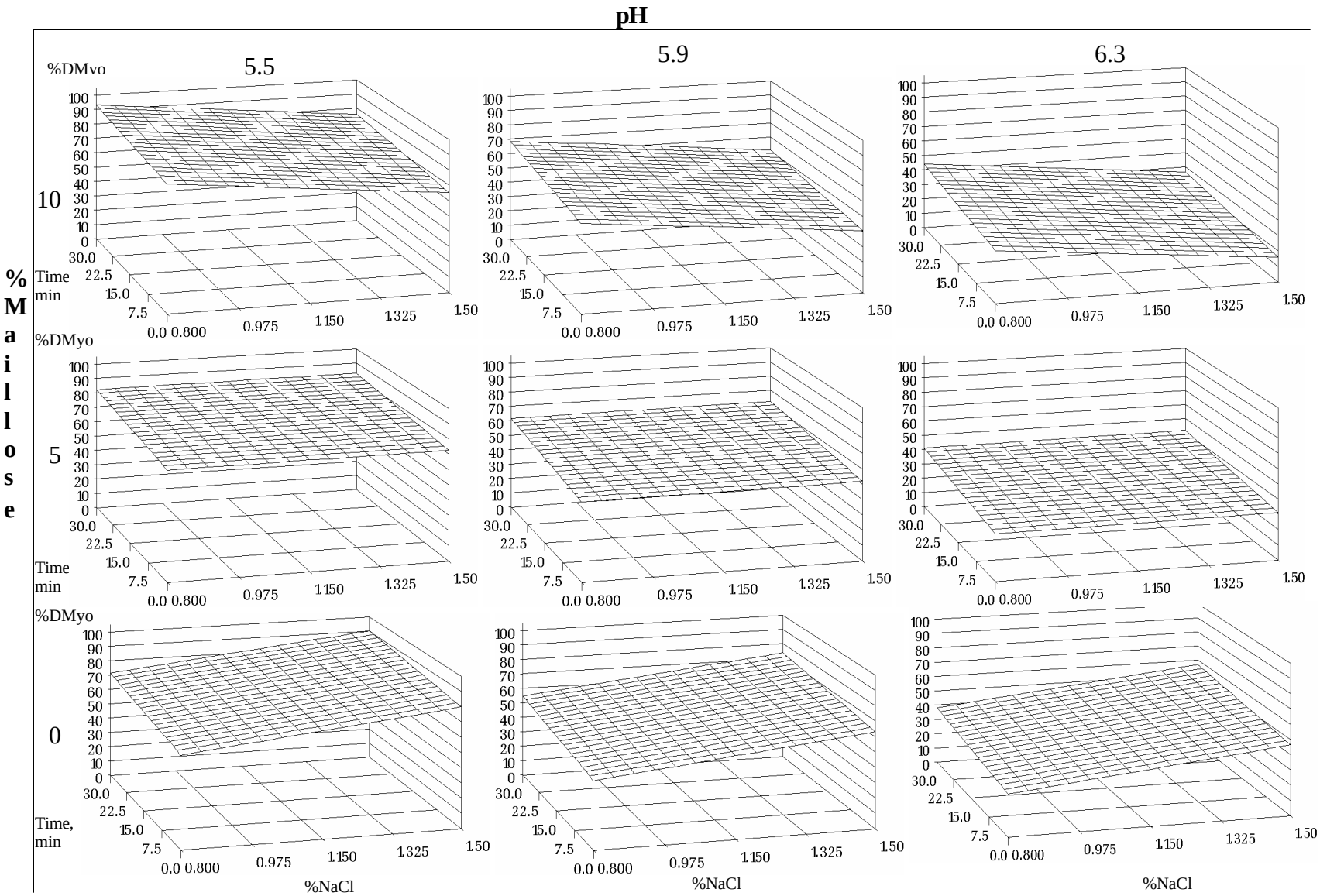

Figure 2.3. Response Surface of myoglobin denaturation at 80^{o}C from model equation of deoxymyoglobin in Table. 2.1.

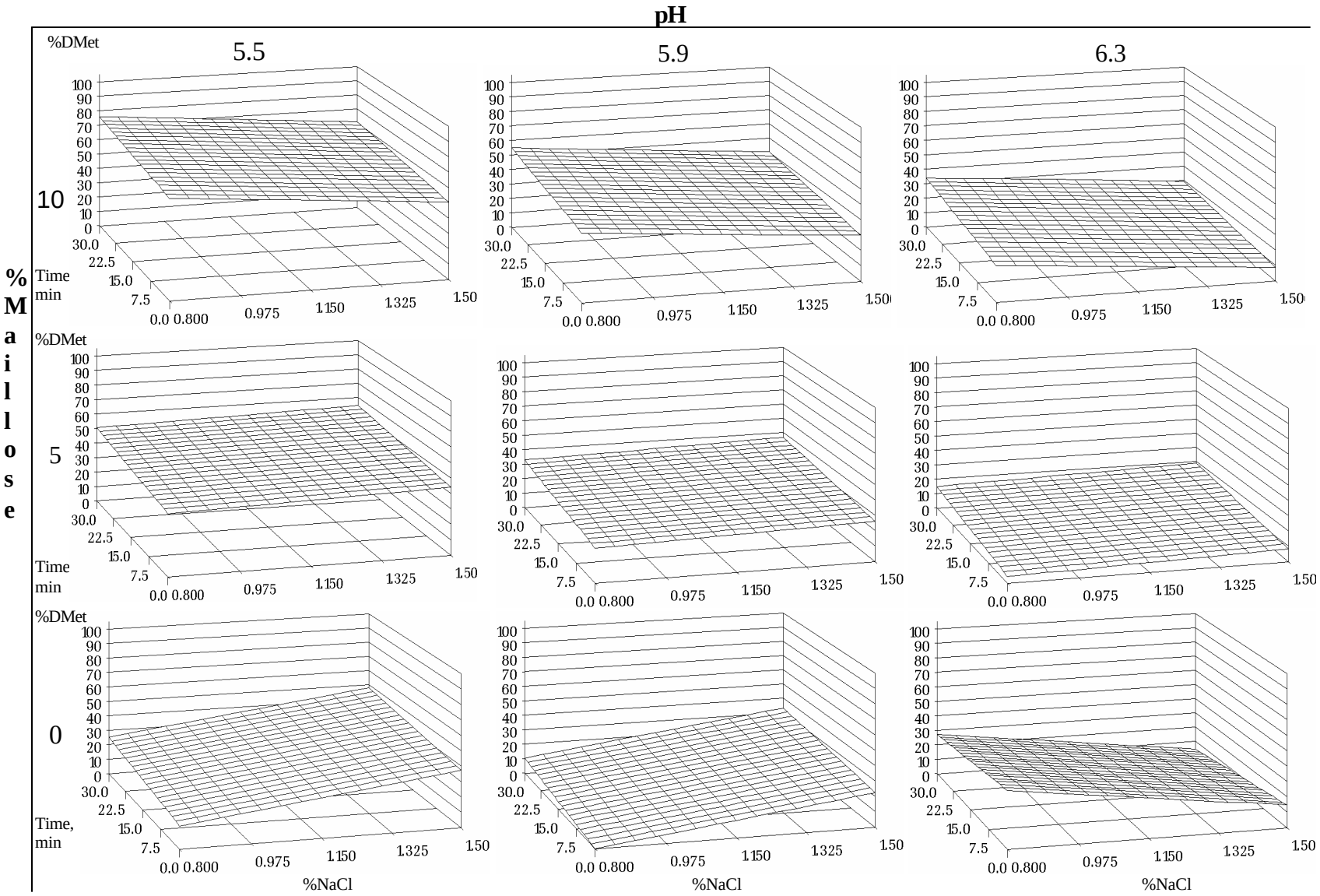

Figure 2.4. Response Surface of metmyoglobin derivative denaturation at 60°C from model equation of metmyoglobin in Table. 2.2.

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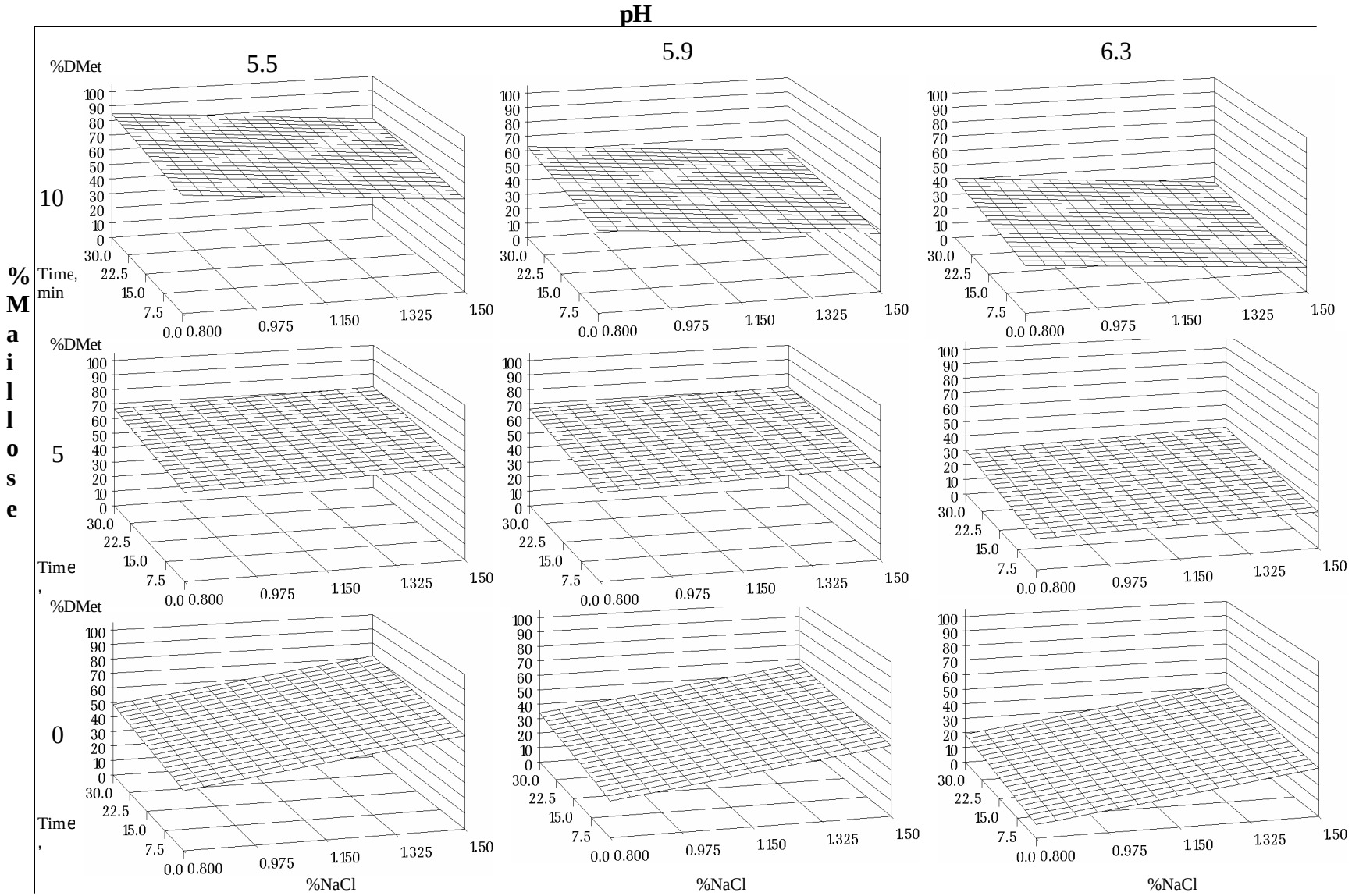


Figure 2.5. Response Surface of metmyglobin derivative denaturation at 70°C from model equation of metmyoglobin in Table 2.2.

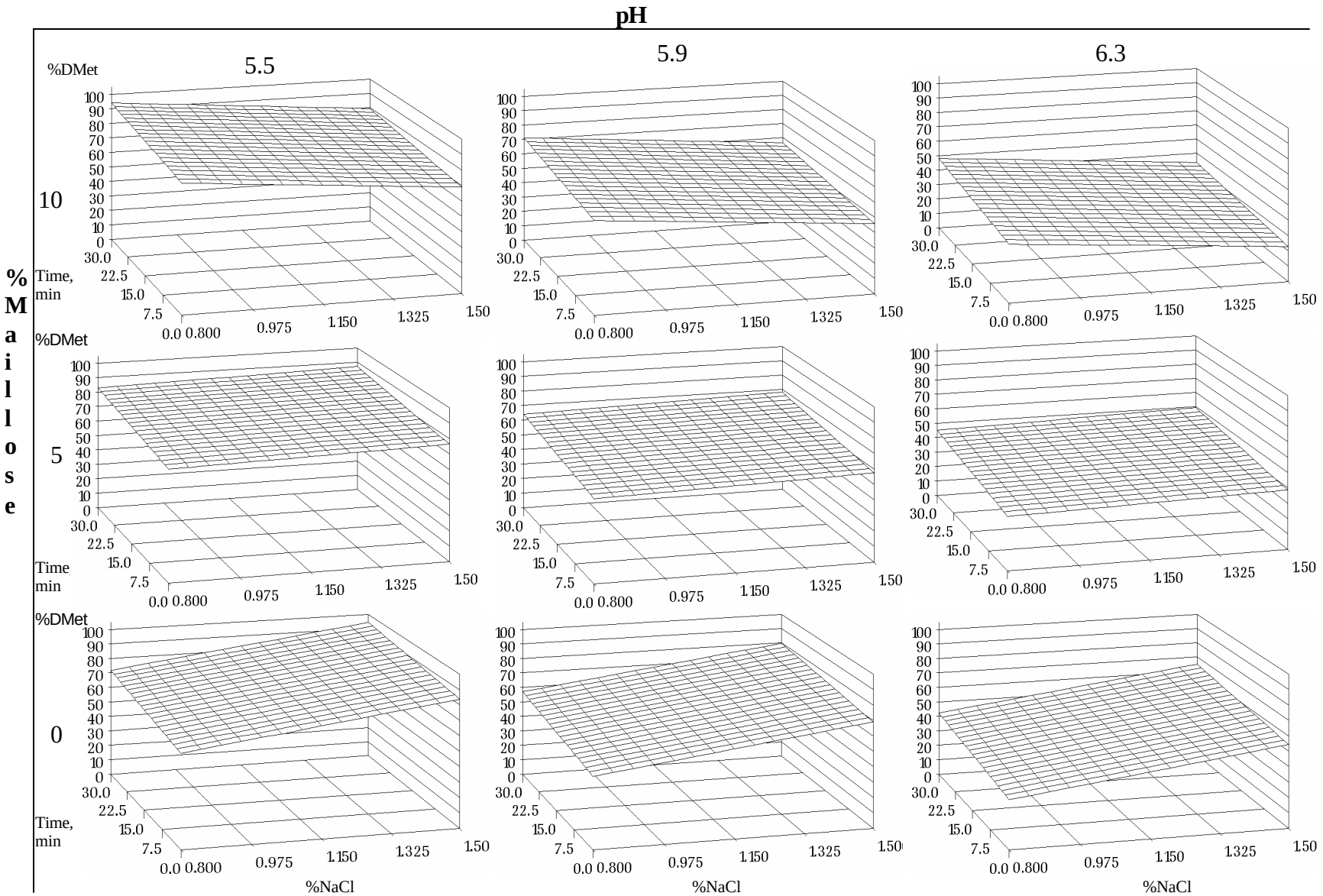


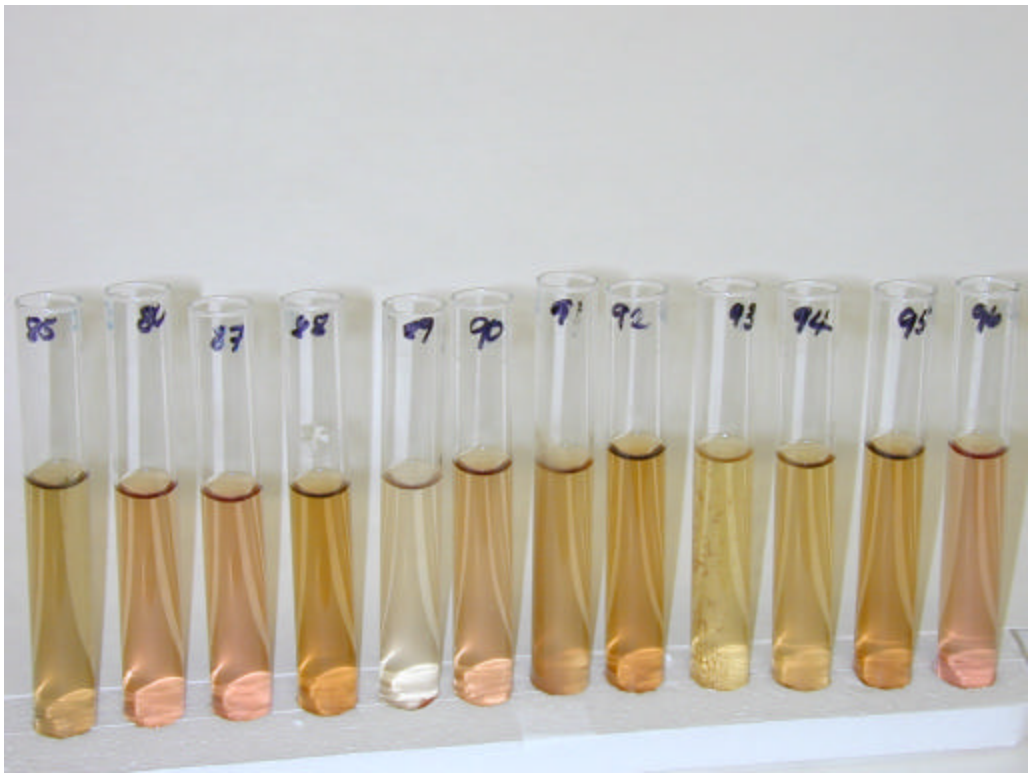
Figure 2.6. Response Surface of metmyoglobin derivative denaturation at 80°C from model equation of metmyoglobin in Table 2.2.

Figure 2.7. a and b) Myoglobin derivative at different NaCl, pH, and Maillose concentration heated to different temperatures and time of exposure.

Legend:

85) 0 min, 60°C, 5.5pH, 0.80%NaCl, 5%Maillose
86) 10 min, 60°C, 5.9pH, 1.15%NaCl, 0%Maillose
87) 20 min, 60°C, 6.3pH, 0.80%NaCl, 0%Maillose
88) 30 min, 60°C, 6.3pH, 1.50%NaCl, 10%Maillose
89) 10 min, 70°C, 5.5pH, 0.80%NaCl, 0%Maillose
90) 30 min, 70°C, 5.9pH, 0.80%NaCl, 0%Maillose
91) 0 min, 70°C, 5.9pH, 0.80%NaCl, 10%Maillose
92) 10 min, 70°C, 6.3pH, 1.15%NaCl, 10%Maillose
93) 30 min, 80°C, 5.5pH, 1.15%NaCl, 10%Maillose
94) 20 min, 80°C, 5.9pH, 1.15%NaCl, 0%Maillose
95) 10 min, 80°C, 6.3pH, 0.80%NaCl, 5%Maillose
96) 0 min, 80°C, 6.3pH, 1.15%NaCl, 0%Maillose

a)



b)

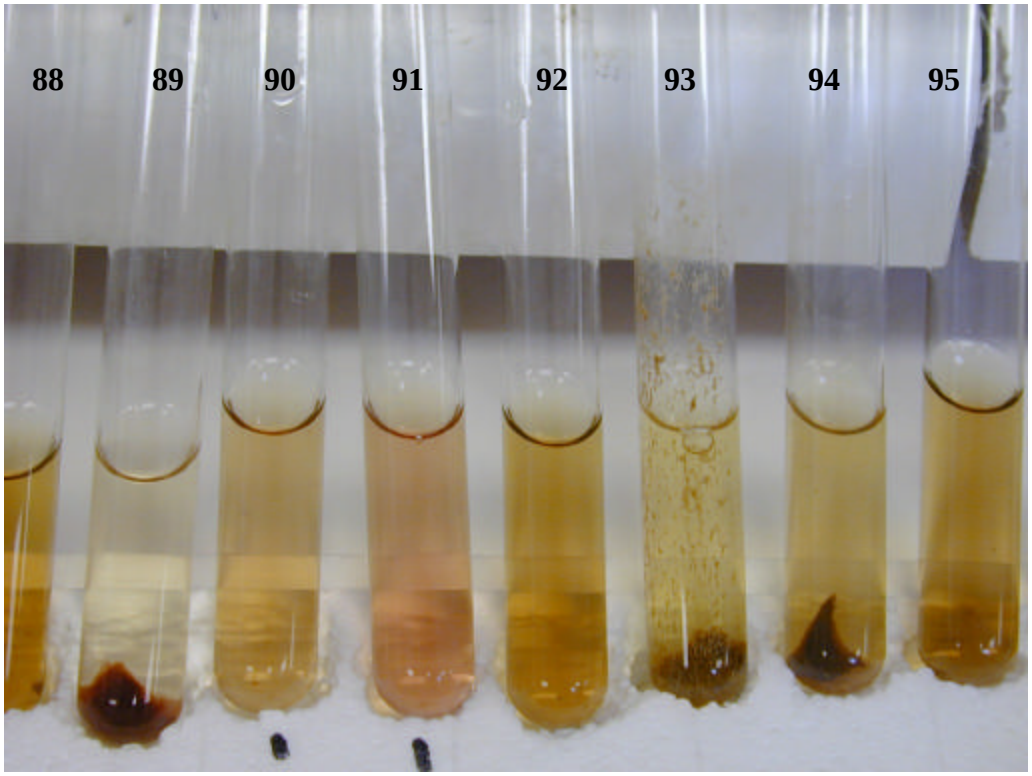
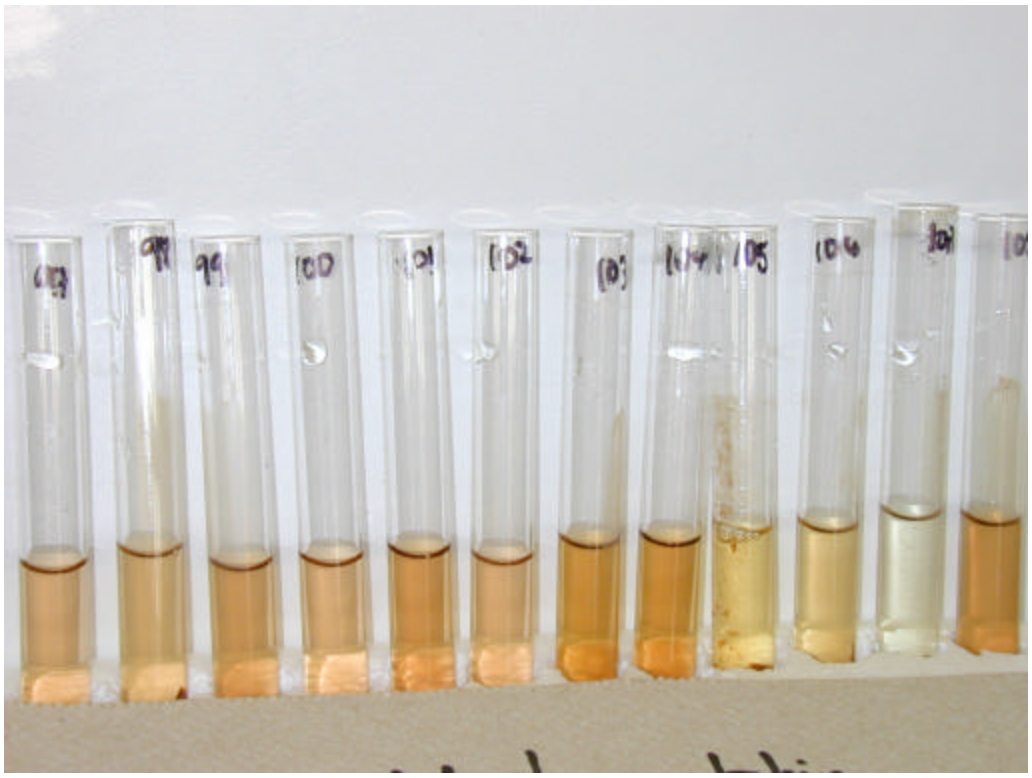


Figure 2.8. a and b) Metmyoglobin derivative at different NaCl, pH, and Maillose concentration heated to different temperatures and time of exposure.

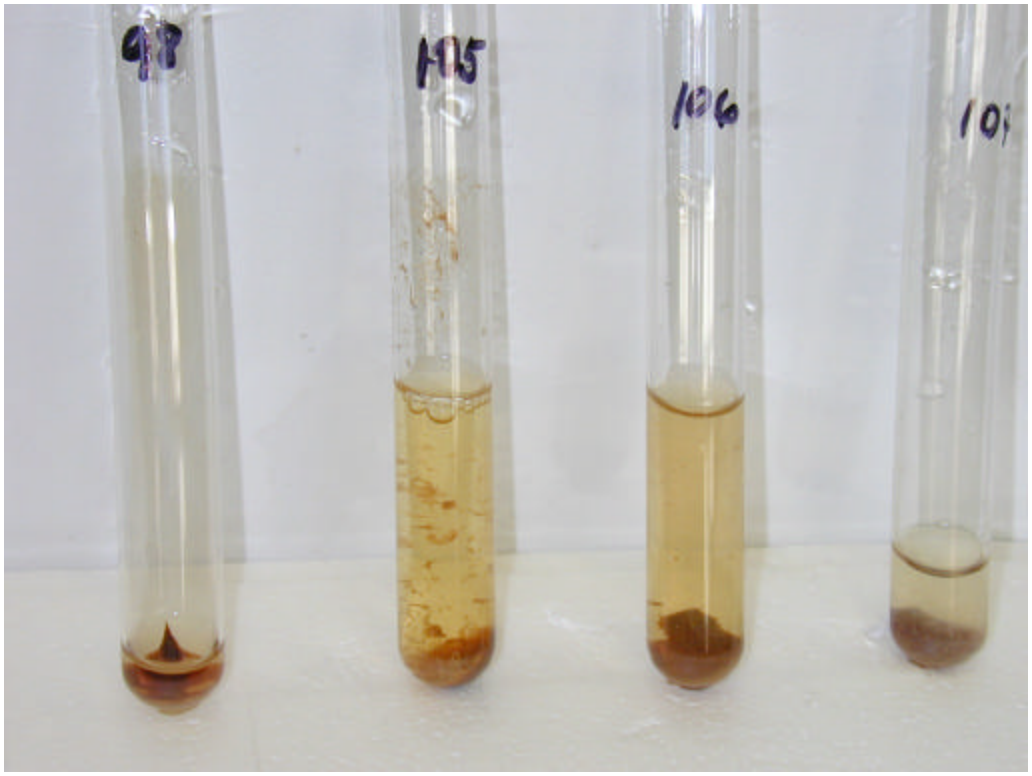
Legend:

97) 20 min, 60°C, 5.5pH, 1.15%NaCl, 0%Maillose
98) 10 min, 60°C, 5.5pH, 1.50%NaCl, 5%Maillose
99) 20 min, 60°C, 5.9pH, 0.80%NaCl, 5%Maillose
100) 30 min, 60°C, 5.9pH, 1.50%NaCl, 0%Maillose
101) 10 min, 60°C, 6.3pH, 0.80%NaCl, 10%Maillose
102) 0 min, 70°C, 5.5pH, 0.80%NaCl, 0%Maillose
103) 20 min, 70°C, 5.9pH, 1.50%NaCl, 10%Maillose
104) 30 min, 70°C, 6.3pH, 1.15%NaCl, 5%Maillose
105) 30 min, 80°C, 5.5pH, 0.80%NaCl, 10%Maillose
106) 10 min, 80°C, 5.9pH, 1.15%NaCl, 5%Maillose
107) 20 min, 80°C, 6.3pH, 1.50%NaCl, 0%Maillose
108) 0 min, 80°C, 6.3pH, 1.50%NaCl, 5%Maillose

a)



b)



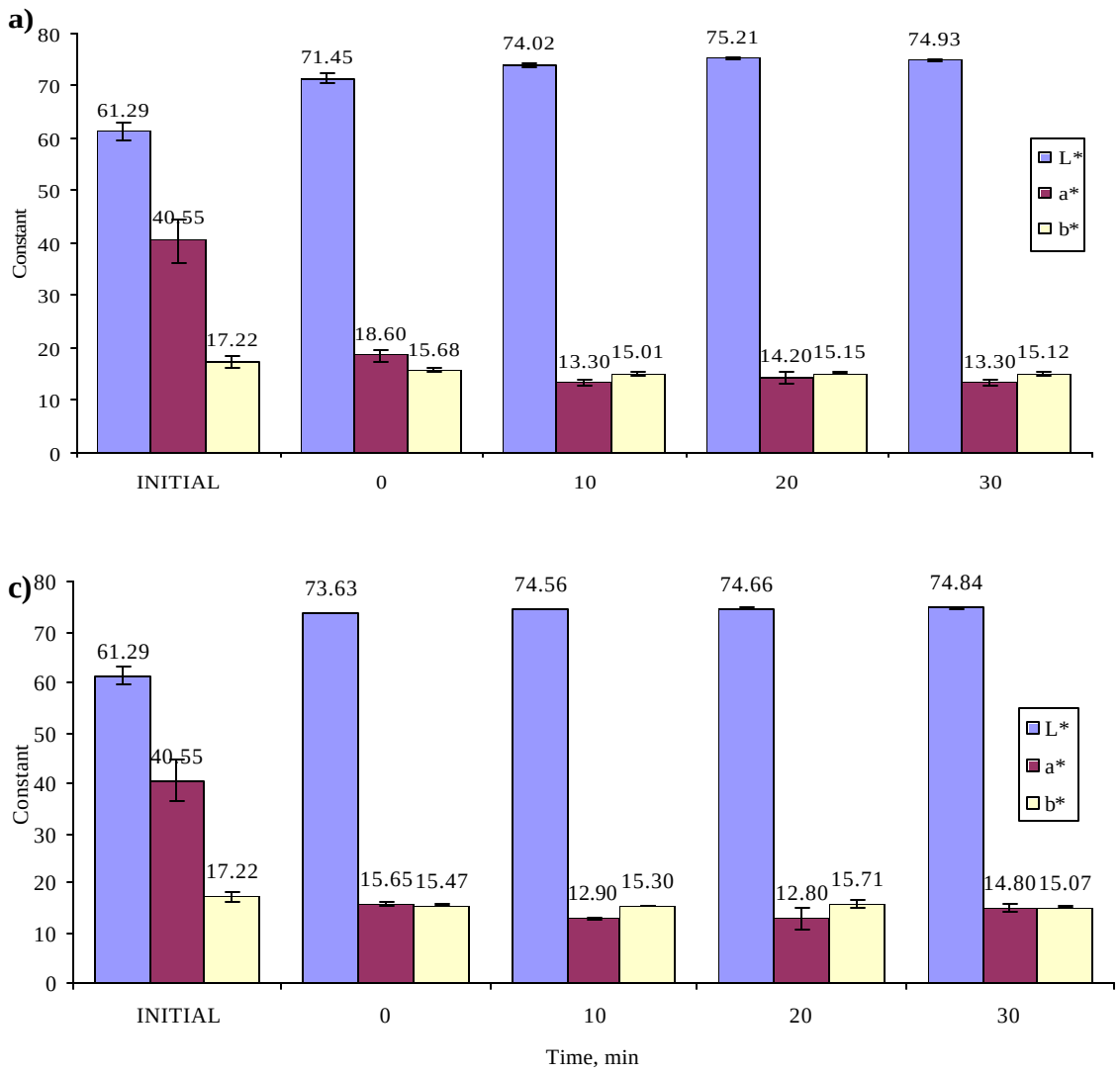


Figure 2.9. Cooked chicken sausage (pH 5.5, 1.5% NaCl, 0% Maillose) CIE L*, a* x 0.1), and b* values profile heated at different times and temperatures. a) 60°C b) 70°C c) 80°C. Pink color was obviously observed in sample 60°C with time 0 min.

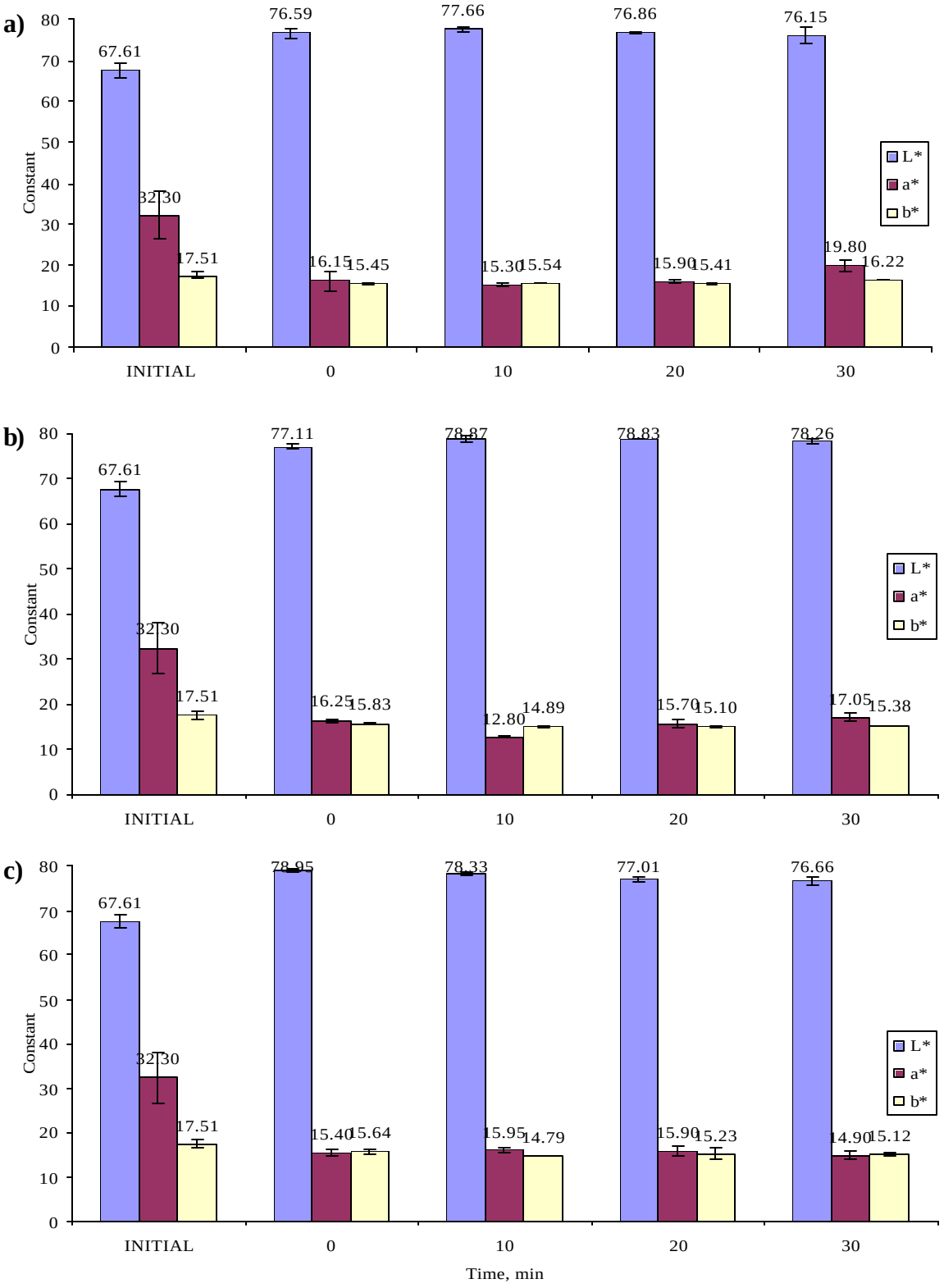


Fig. 2.10. Cooked chicken sausage (pH 5.5, 0.5% NaCl; 0% Maillose) CIE L*, a* x 0.1 and b* values profile heated at different times and temperatures. a) 60^{o}C b) 70^{o}C c) 80^{o}C. No pink color was obviously observed in all heated meat samples.

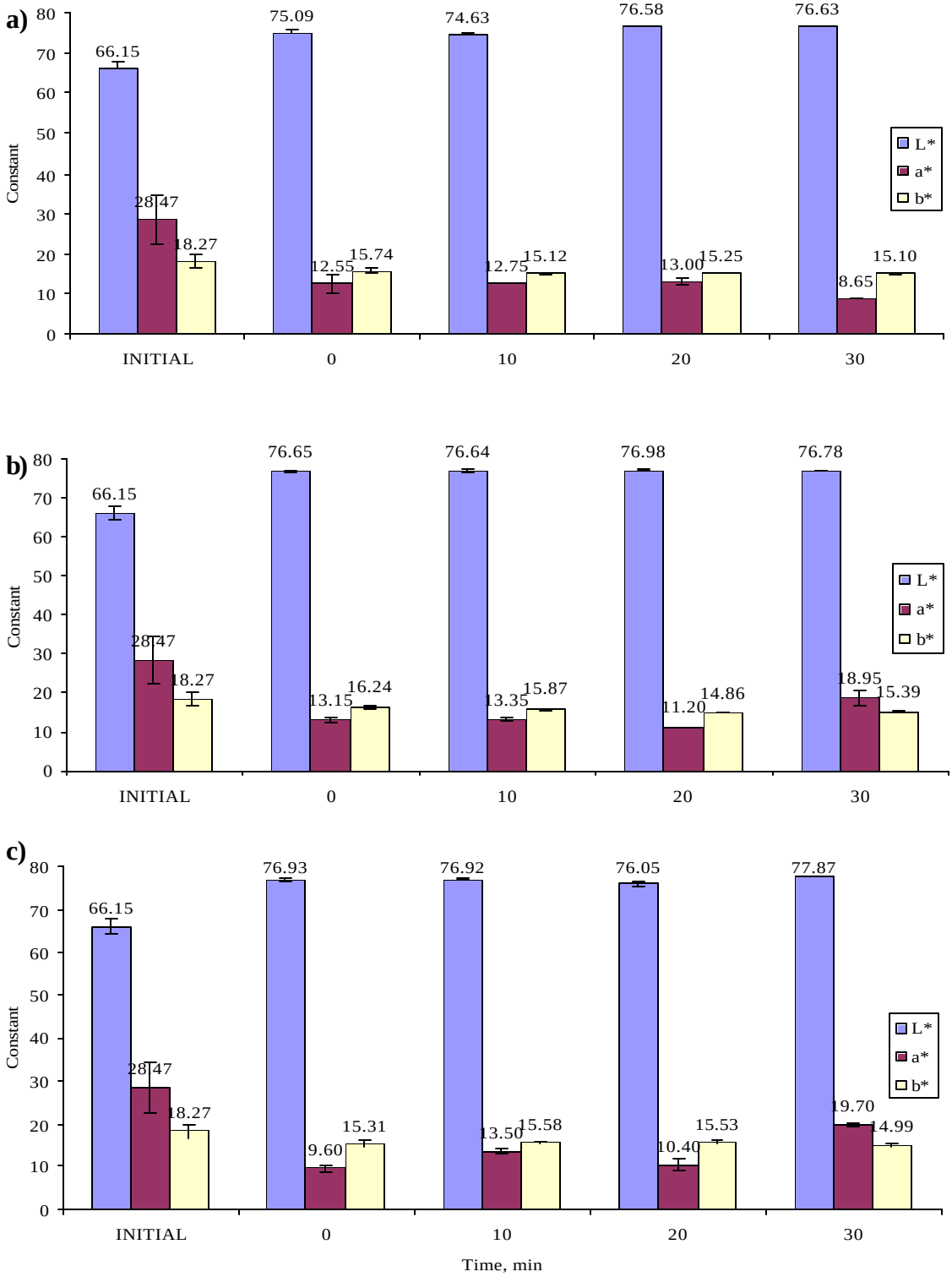
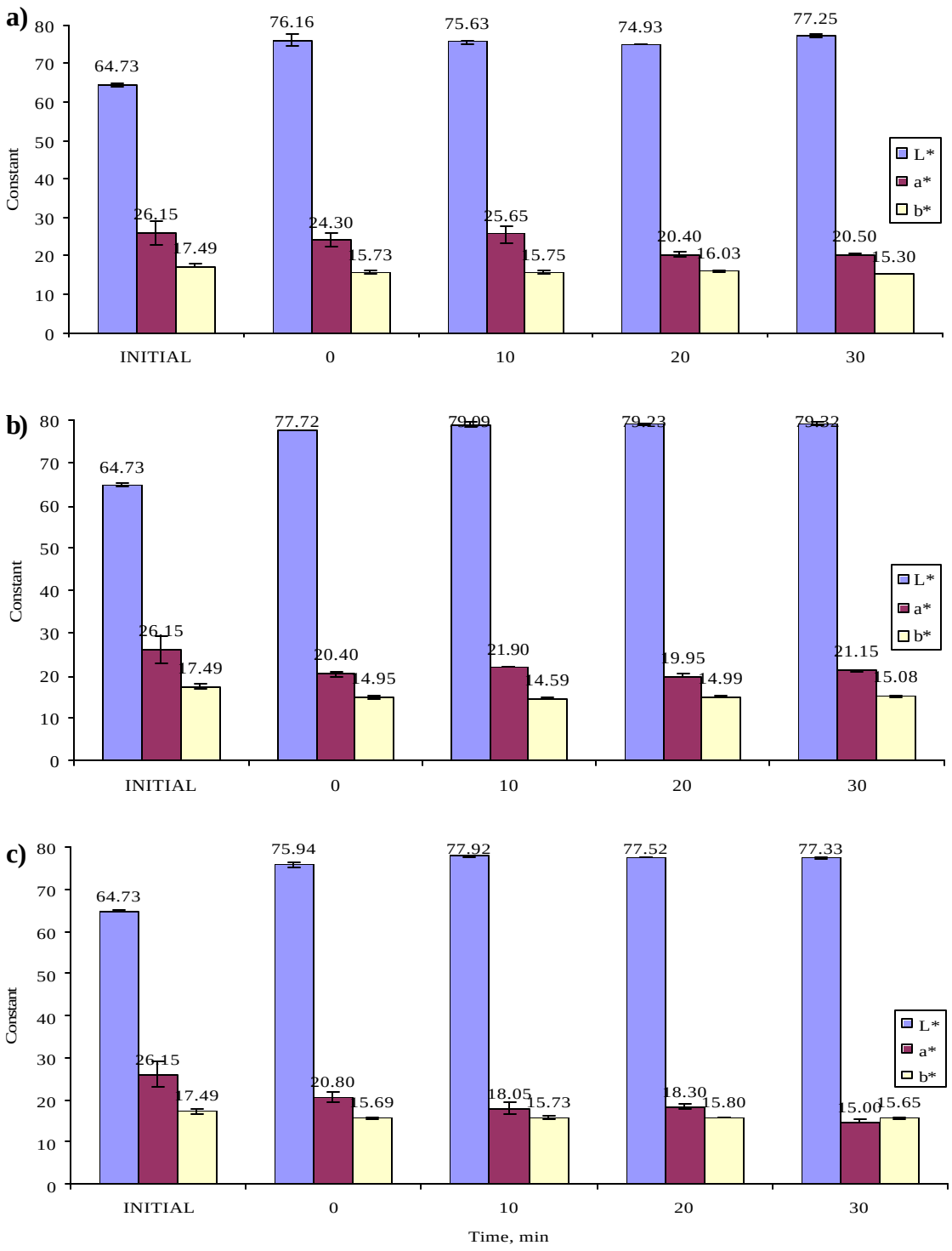


Figure 2.11. Cooked chicken sausage (pH 5.5, 1.5% NaCl; 0.2%Maillose) CIE L*, a*x 0.1 and b* values profile heated at different times and temperatures. a) 60^{o}C b) 70^{o}C c)80^{o}C. No pink color was obviously observed in all heated samples.



Figures 2.12. Cooked chicken sausage (pH 5.5, 0.5% NaCl; 0.2%Maillose) CIE L*, a*x 0.1 and b* values profile heated at different times and temperatures. a) 60°C b) 70°C c) 80°C. Pink color was observed in samples heated at 60 and 70°C up to 30 min. No pink color was obviously observed in samples heated at 80°C except at time 0min.

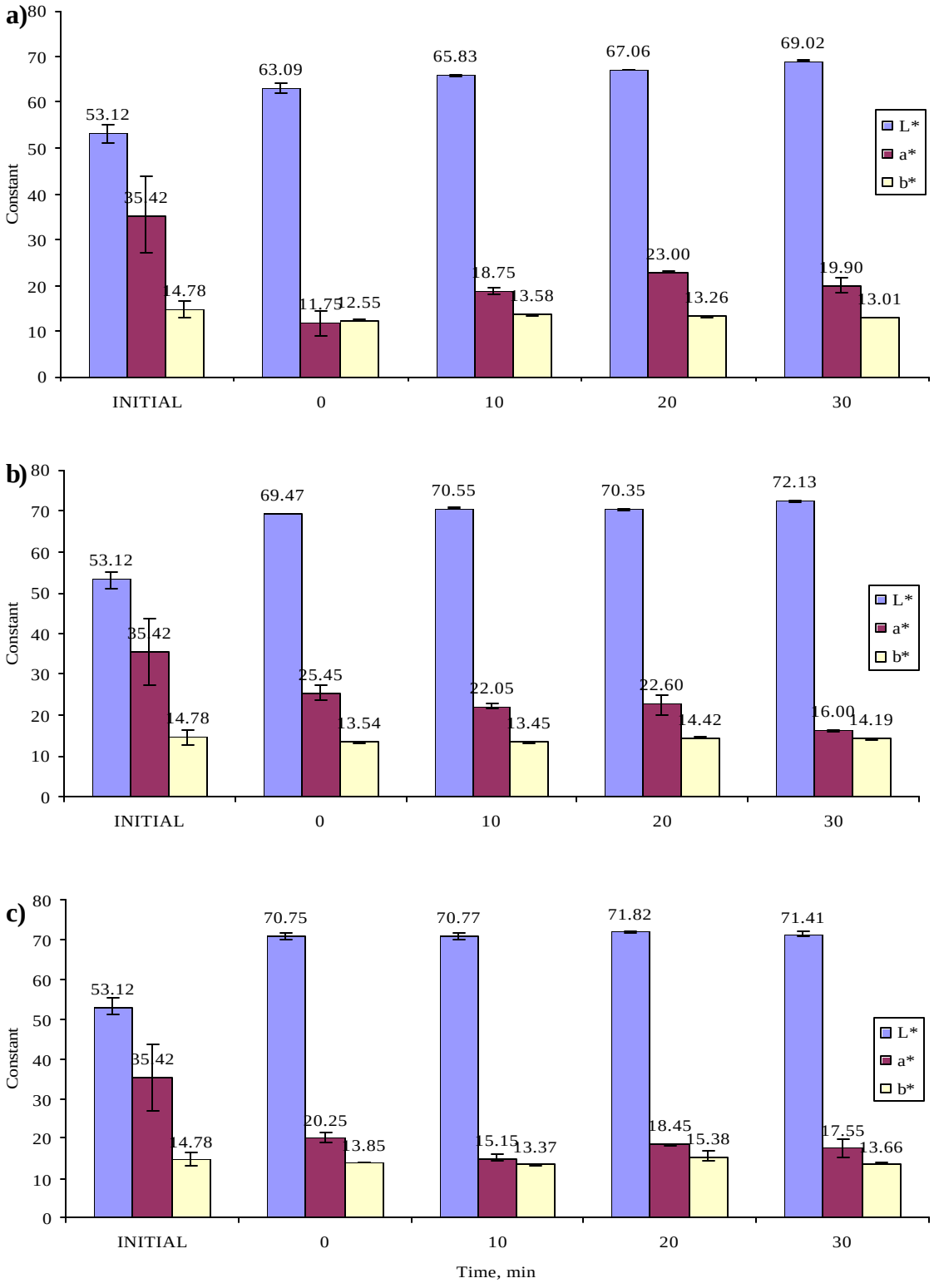


Figure 2.13 Cooked chicken sausage (pH 6.3, 1.5% NaCl; 0%Maillose) CIE L*, a*x 0.1 and b* values profile heated at different times and temperatures. a) 60^{o}C b) 70^{o}C c) 80^{o}C. Pink color was obviously observed in all heated samples.

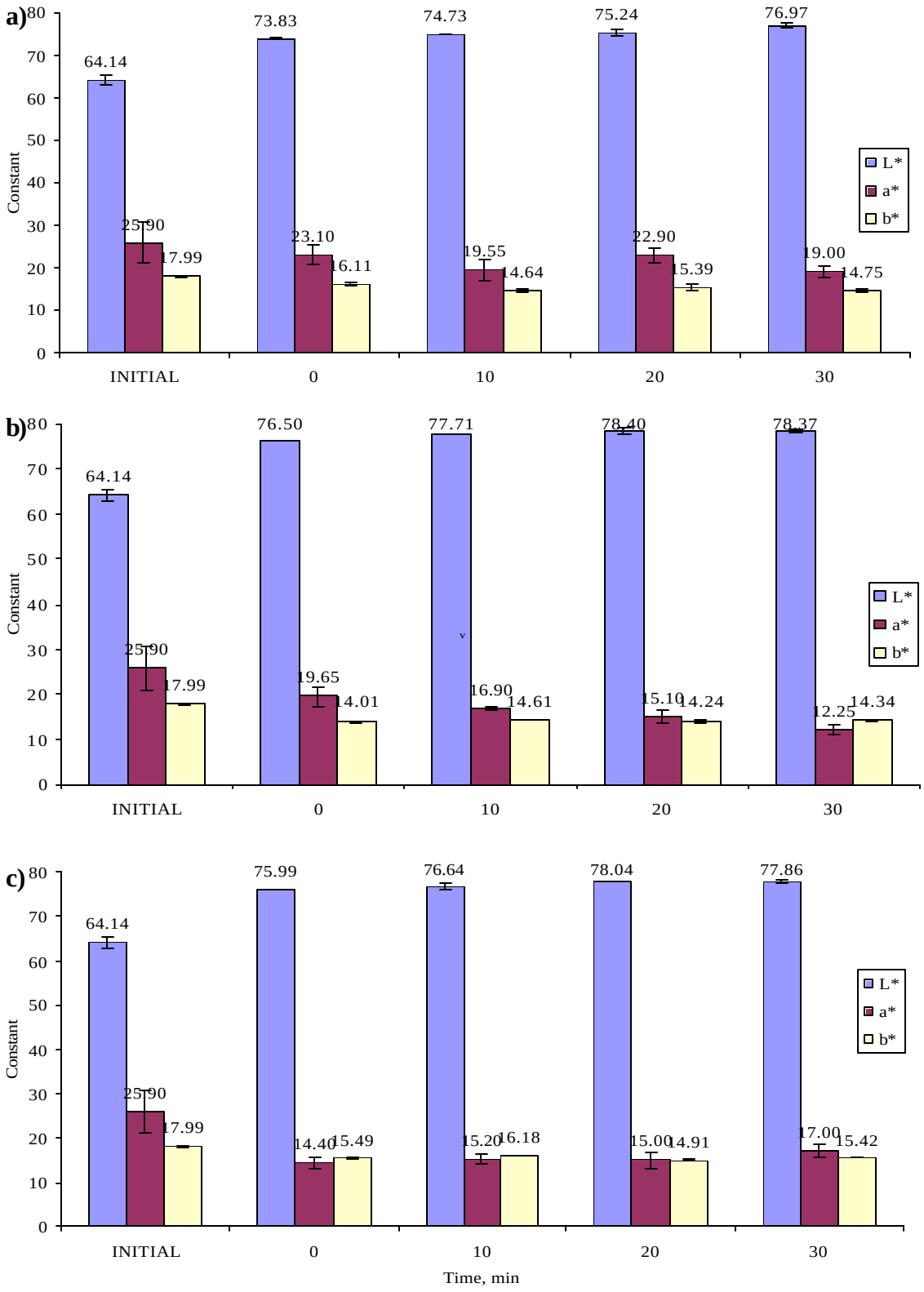


Fig. 2.14. Cooked chicken sausage (pH 6.3, 0.5% NaCl; 0%Maillose) CIE L*, a*x 0.1 and b* values profile heated at different times and temperatures. a) 60^{o}C b) 70^{o} c) 80^{o}C. Pink color was obviously observed in samples heated at 60^{o}C only.

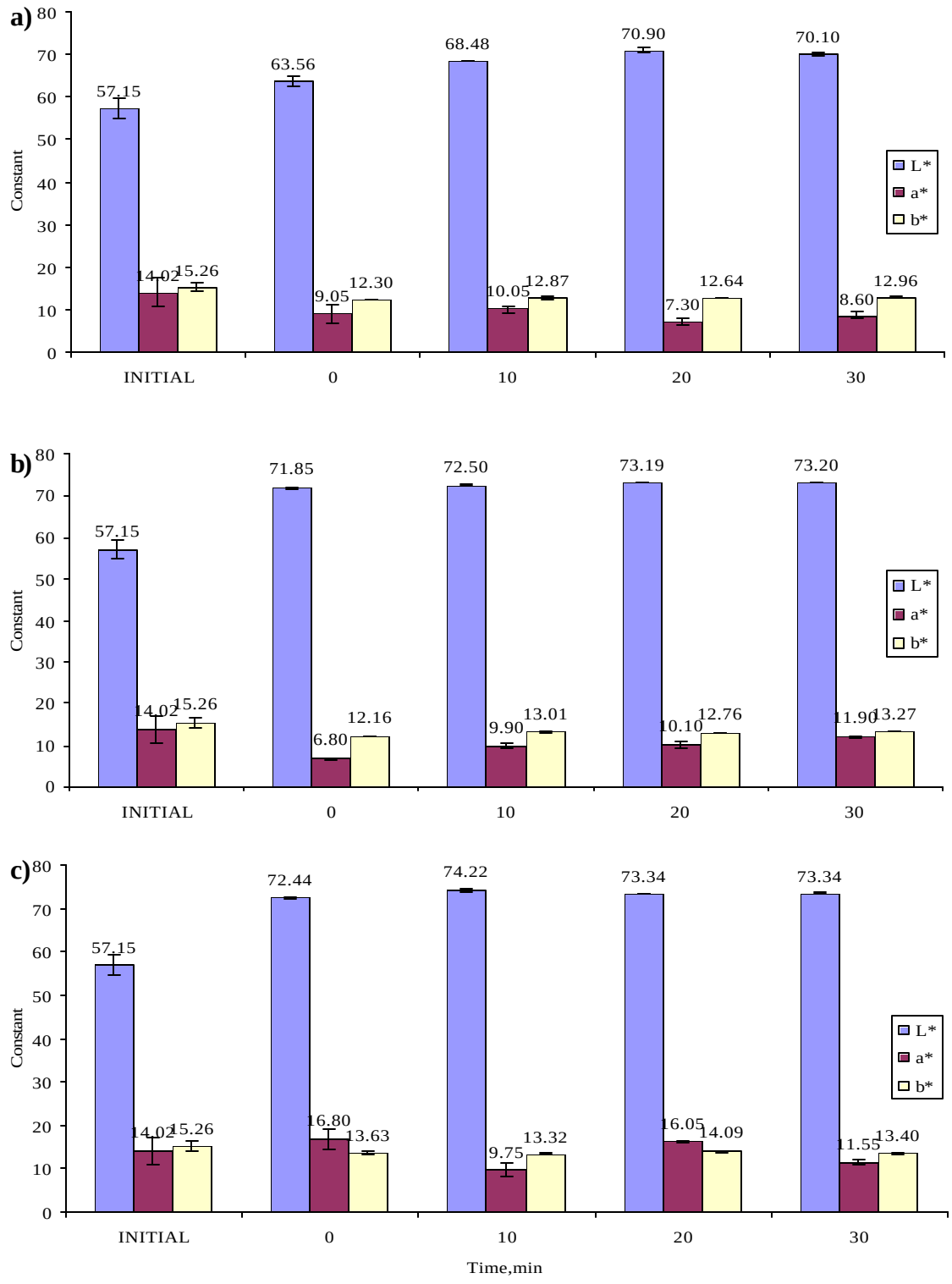


Figure 2.15. Cooked chicken sausage (pH 6.3, 1.5% NaCl; 0.2%Maillose) CIE L*, a* x 0.1 and b* values profile heated at different times and temperatures. a) 60°C b) 70°C c) 80°C. Pink color was obviously observed in samples heated at 60 and 70°C. No pink color was obviously observed in samples heated at 80°C except at time 0 min.

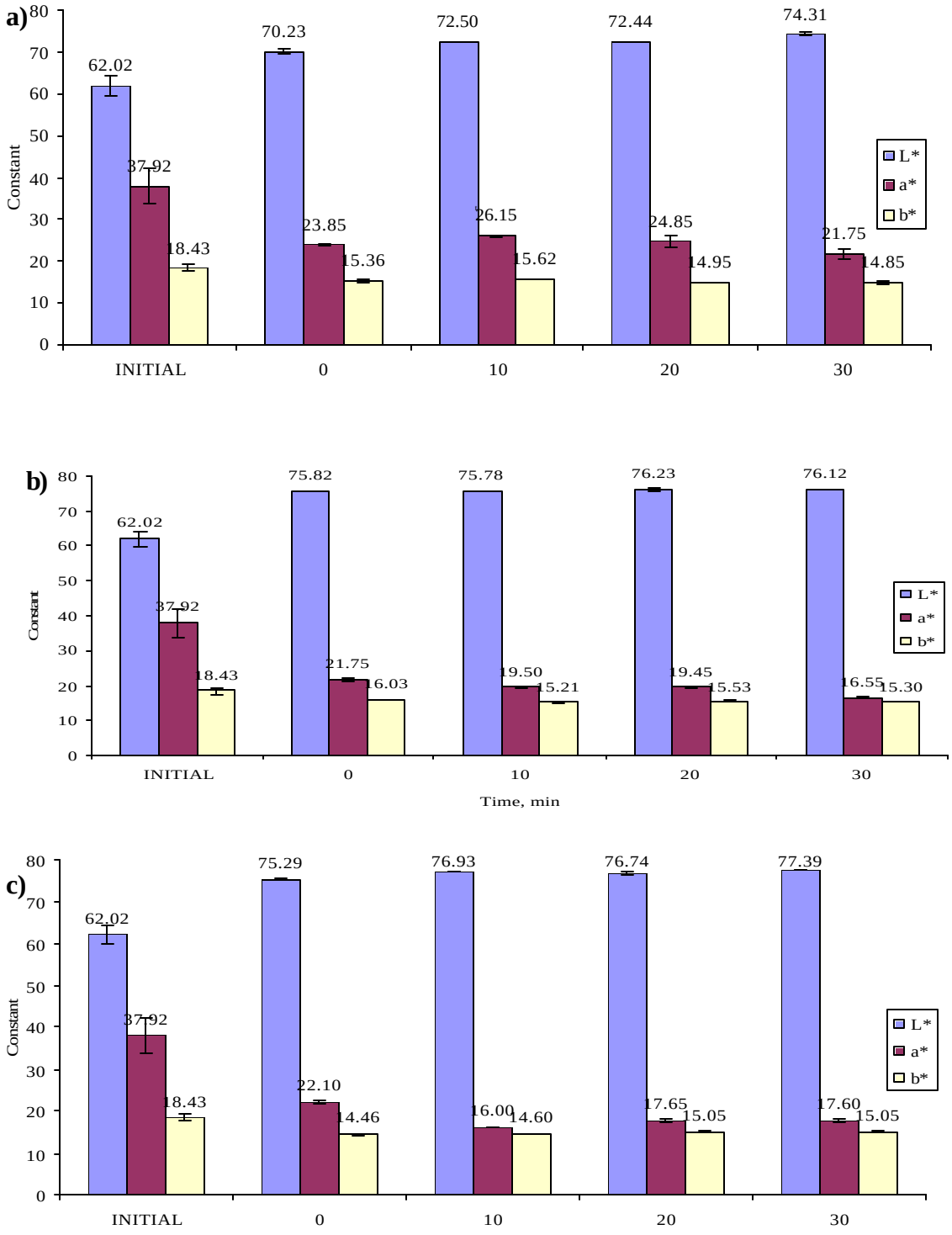


Figure 2.16. Cooked chicken sausage (pH 6.3, 0.5% NaCl; 0.2%Maillosel) CIE L*, a*x 0.1 and b* values profile heated at different times and temperatures. a) 60^{o}C b) 70^{o}C c) 80^{o}C. Pink color was obviously observed in samples heated at 60 and 70^{o}C excluding the sample heated for 30min.

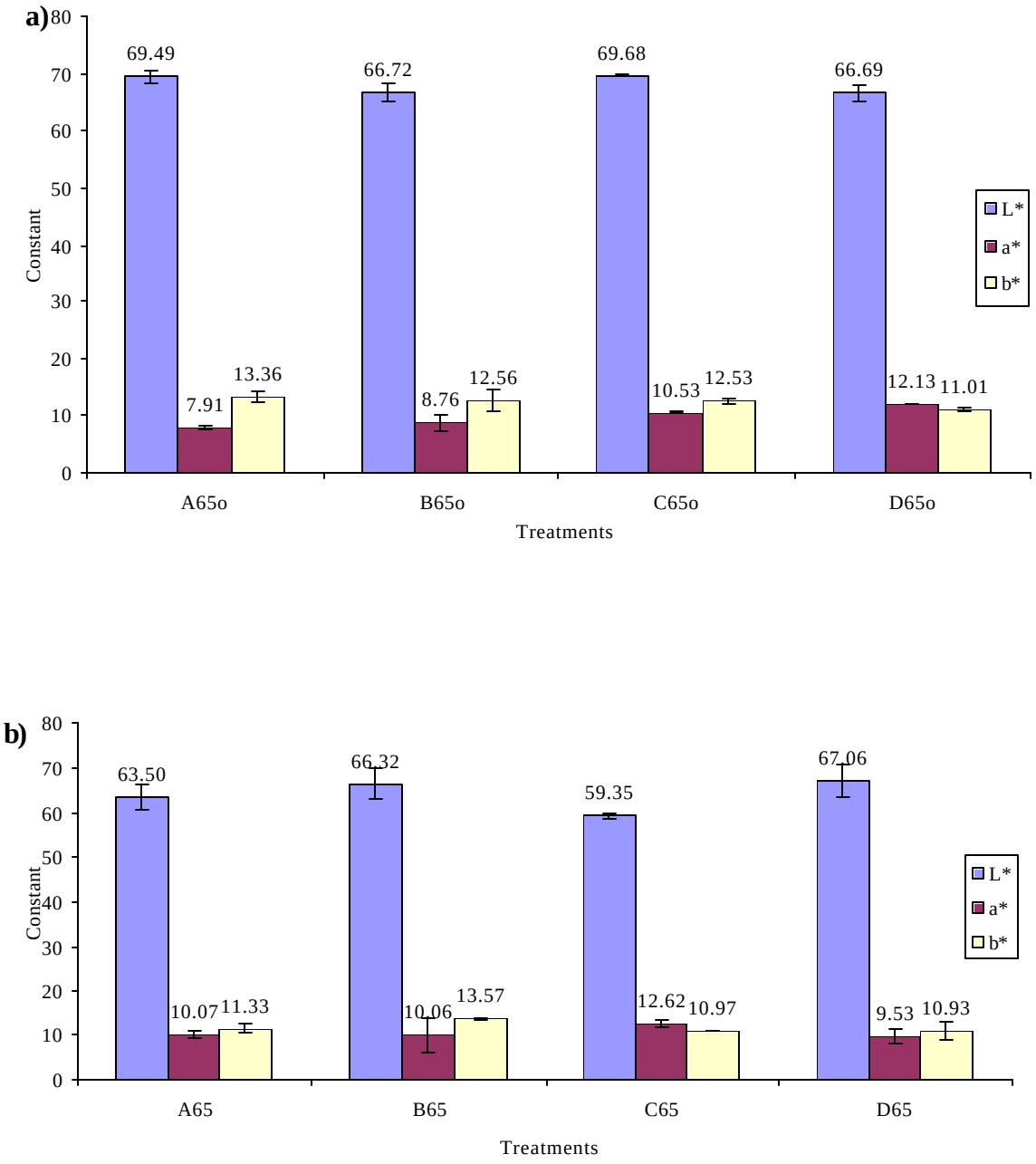


Figure 2.17. CIE L*, a* and b* values of marinated deboned chicken leg quarters heated to an endpoint temperature of 65°C. Marinade composition for Ao, A – 5.5pH, 0.2% Maillose; Bo, B – 5.5pH; Co, C – 6.3pH, 0.2% Maillose; and Do, D – 6.3pH. Ao, Bo, Co, and Do had 0.5% salt while A, B, C, and D had 1.5% salt. Red color was obviously observed in all samples.

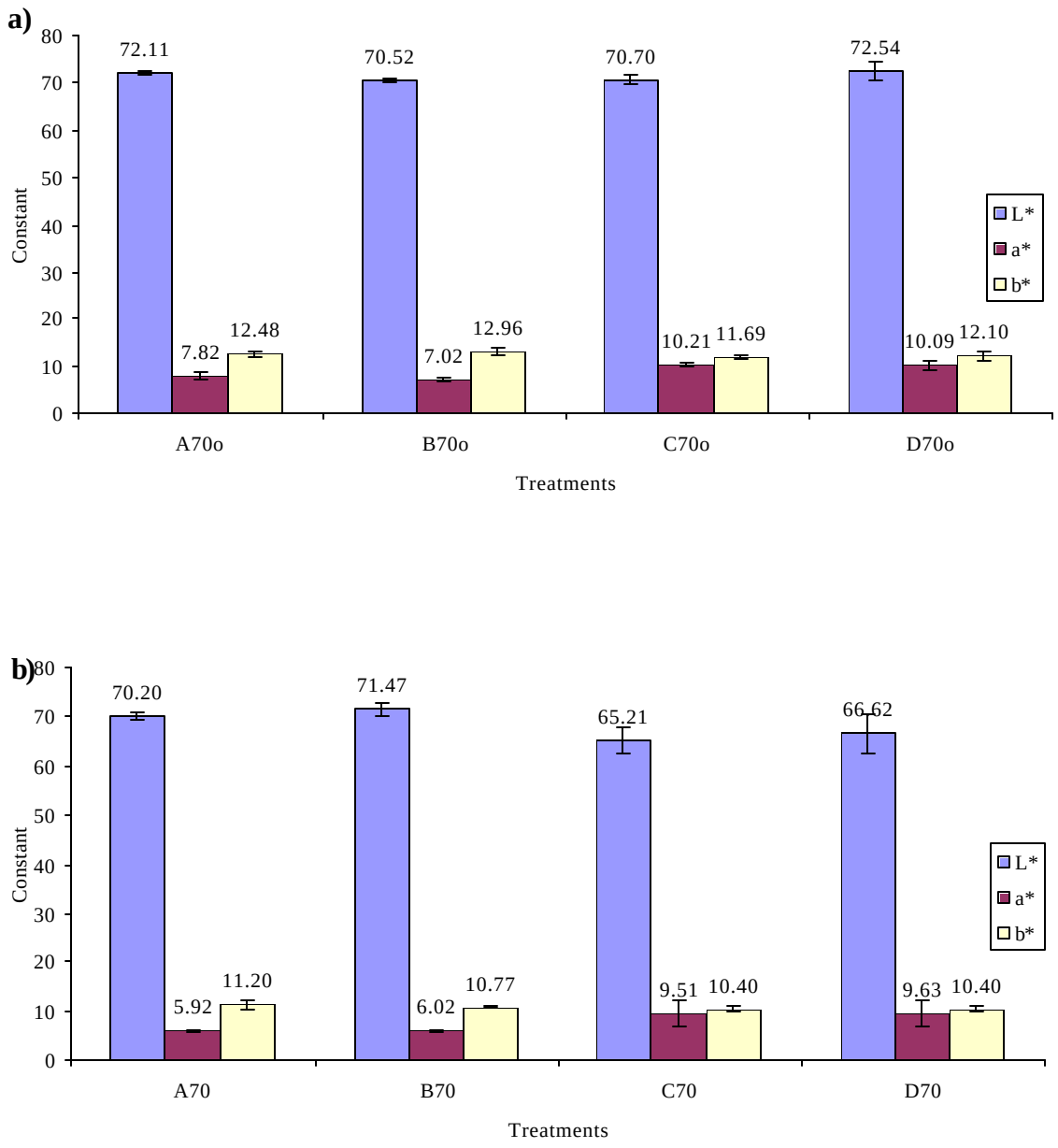


Figure 2.18. CIE L*, a* and b* values of marinated deboned chicken leg quarters heated to an endpoint temperature of 70^{o}C. Marinade composition for Ao, A – 5.5pH, 0.2% Maillose; Bo, B – 5.5pH; Co, C – 6.3pH, 0.2% Maillose; and Do, D – 6.3pH. Ao, Bo, Co, and Do had 0.5% salt while A, B, C, and D had 1.5% salt. Red color was obviously observed in all samples.

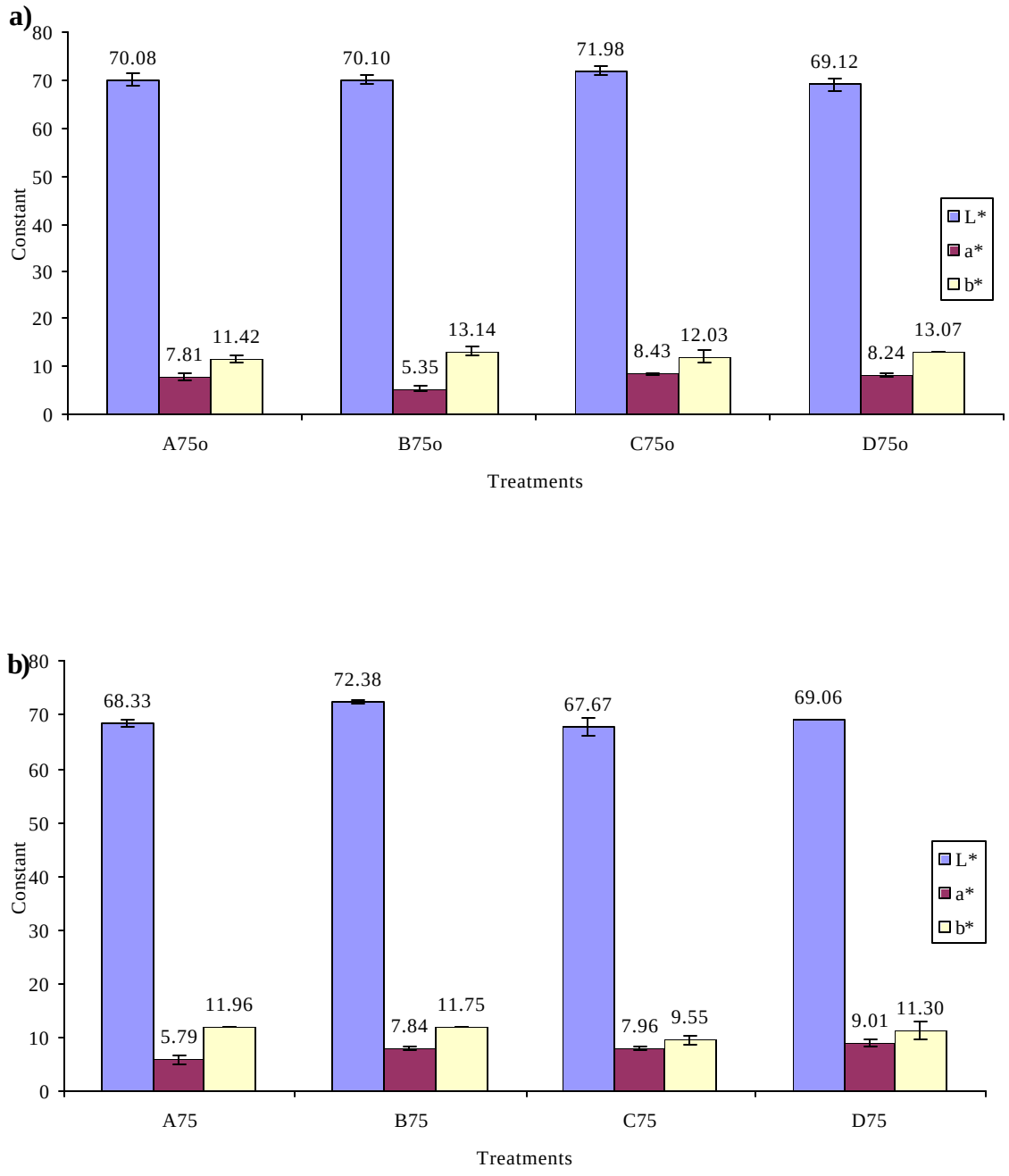


Figure 2.19. CIE L*, a* and b* values of marinated deboned chicken leg quarters heated to an endpoint temperature of 75°C. Marinade composition for Ao, A – 5.5pH, 0.2% Maillose; Bo, B – 5.5pH; Co, C – 6.3pH, 0.2% Maillose; and Do, D – 6.3pH. Ao, Bo, Co, and Do had 0.5% salt while A, B, C, and D had 1.5% salt. Red color was obviously present in all samples.

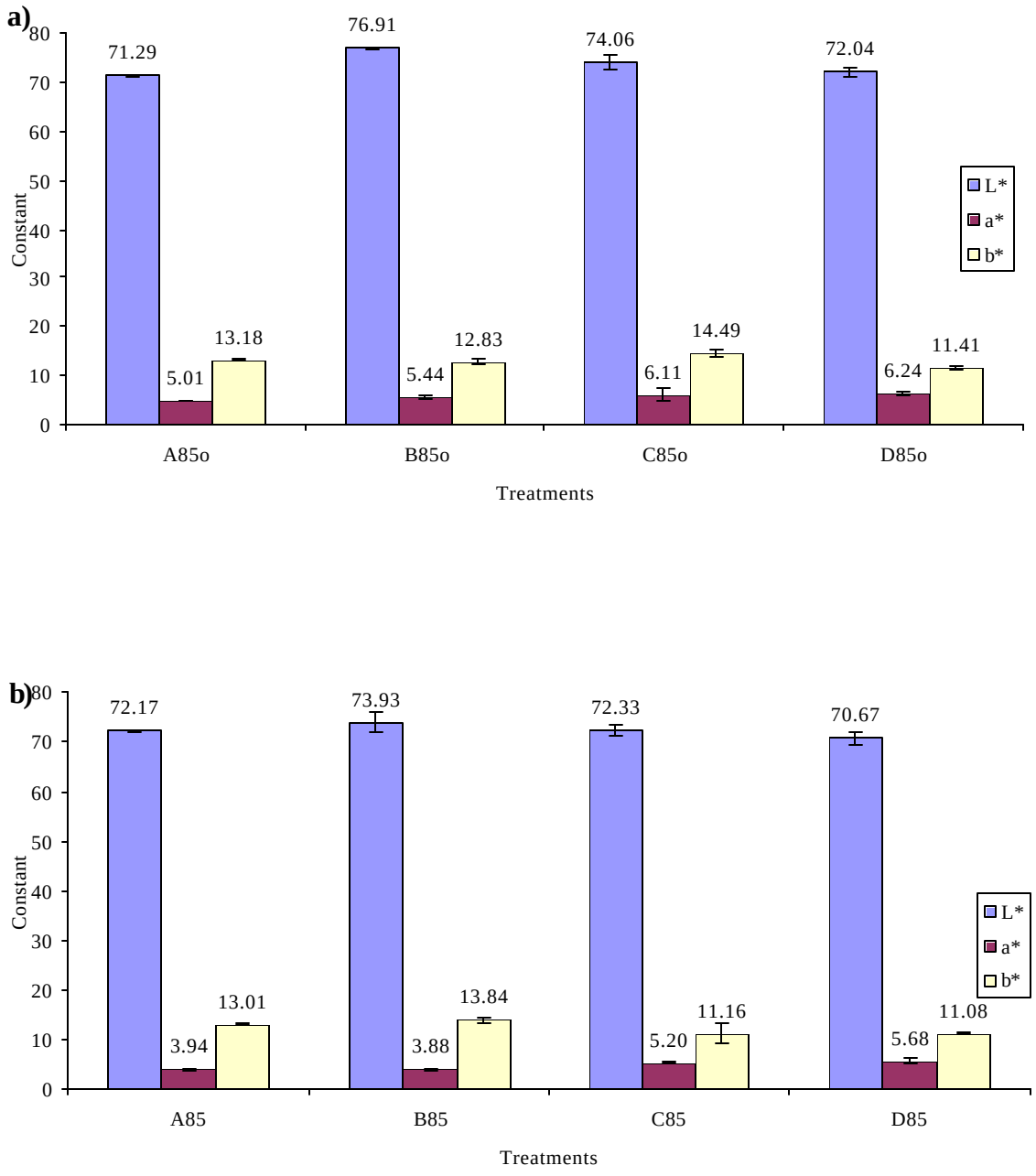
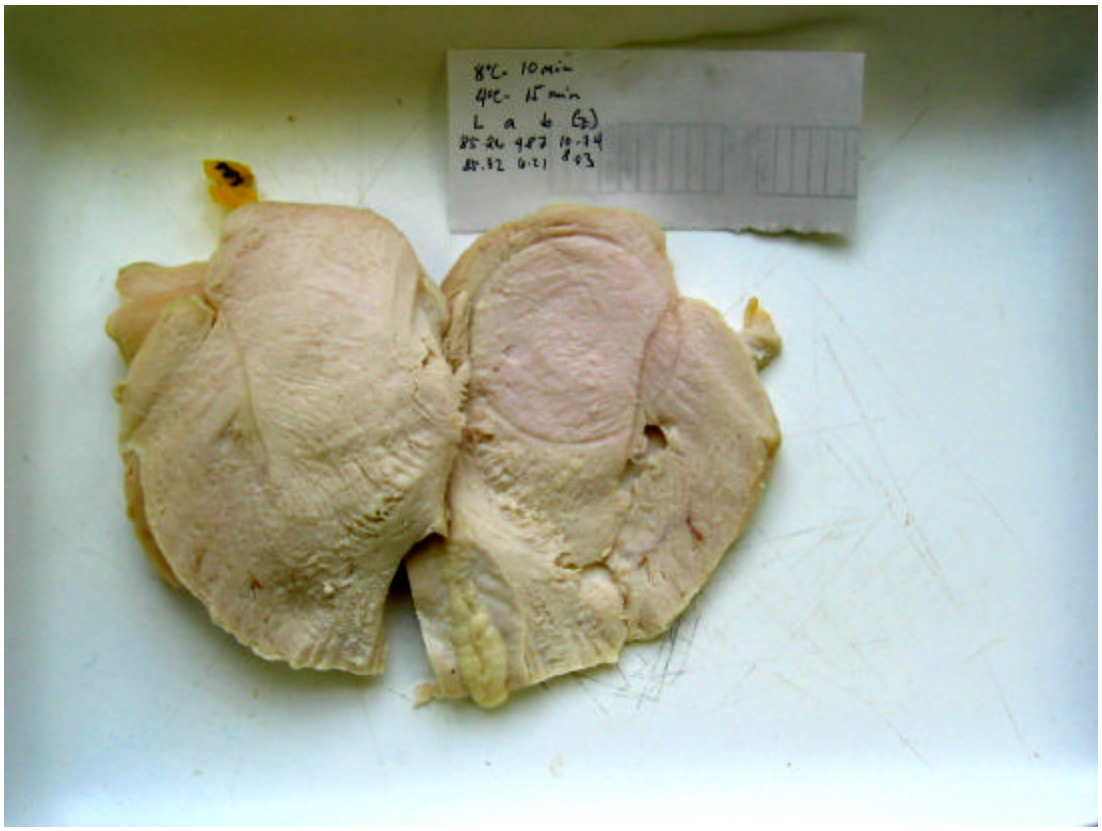


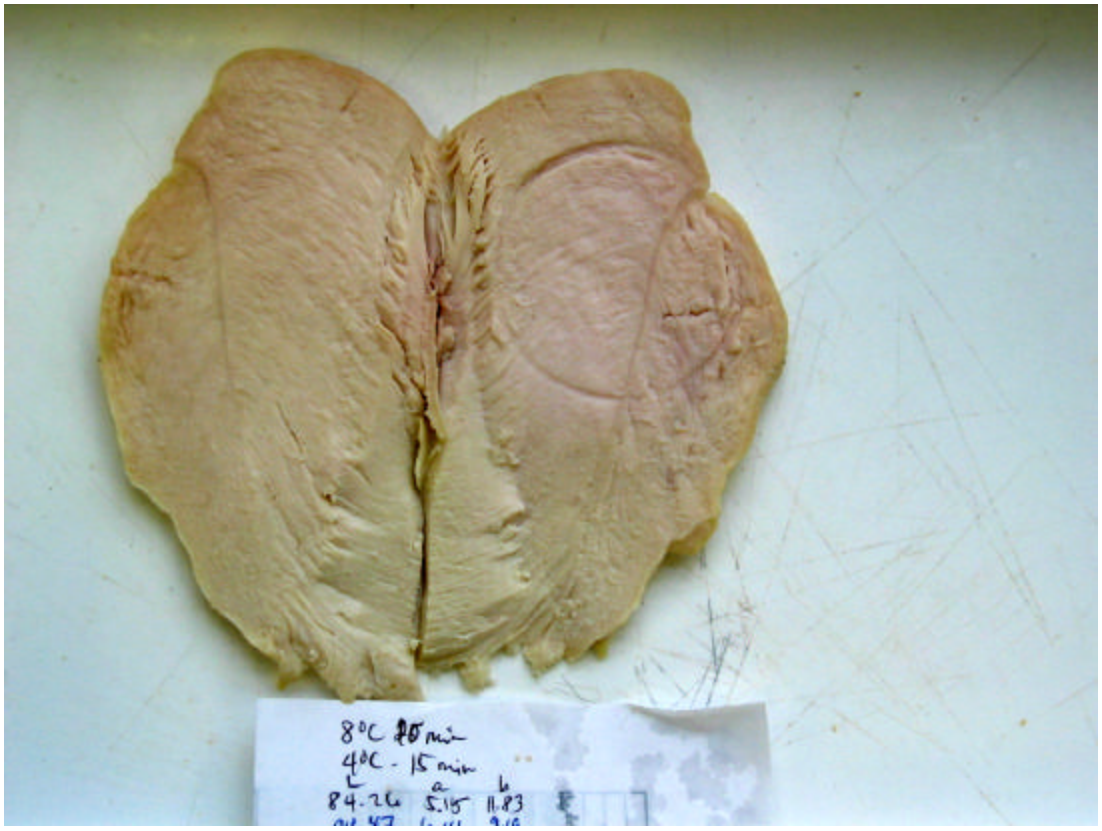
Figure 2.20. CIE L*, a* and b* values of marinated deboned chicken leg quarters heated to an endpoint temperature of 85^{o}C. Marinade composition for Ao, A – 5.5pH, 0.2% Maillose; Bo, B – 5.5pH; Co, C – 6.3pH, 0.2% Maillose; and Do, D – 6.3pH. Ao, Bo, Co, and Do had 0.5% salt while A, B, C, and D had 1.5% salt. Red color was not obviously present in all samples.

Figure 2.21. a, b, c, and d. Showing the color profile of marinated chicken breast with the addition of reducing agent sodium dithionite. Left side of the cooked chicken split breast served as control while the right side breast inside the ring mark was treated with sodium dithionite. Meat samples marination conditions are shown in the photographs. CIE L*,a* and b* values of the chicken samples before and after sodium dithionite treatment are shown in a and c photographs. Photographs b and d displays only the CIE L*, a* b* values of breast fillet before sodium dithionite addition. The samples were stored for 5 days.

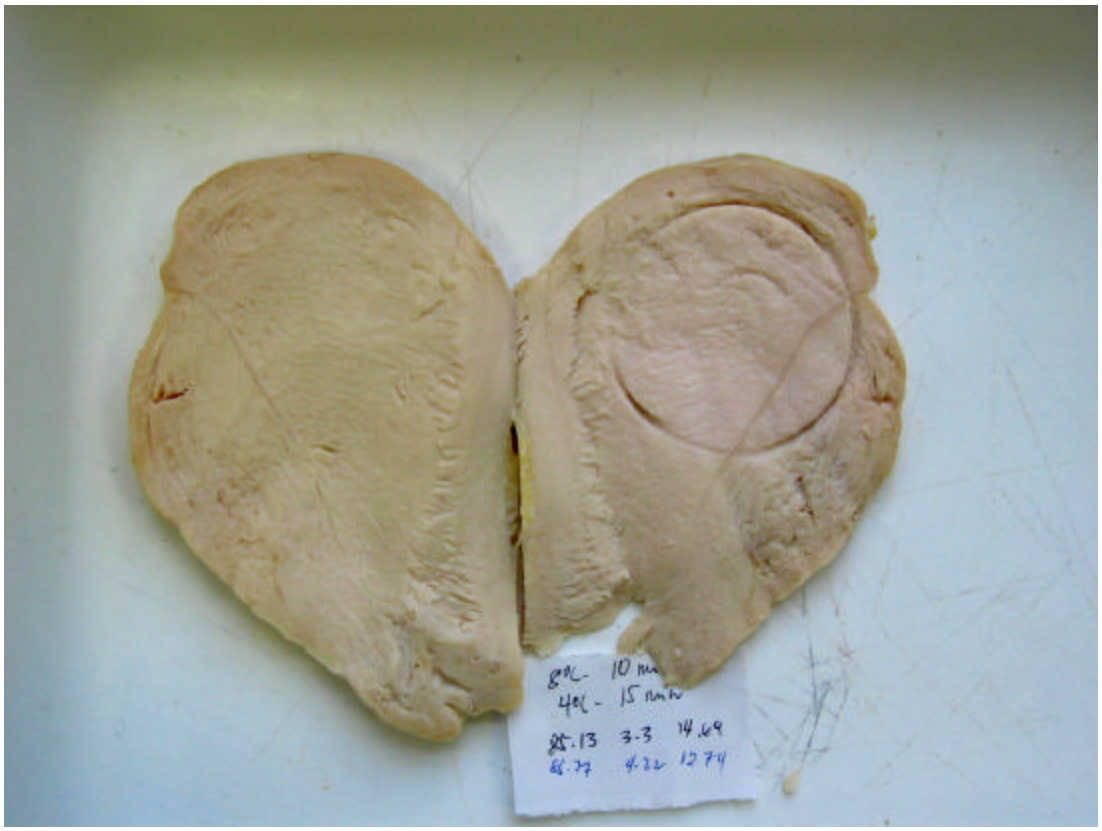
a)


8°C- 10 min
4°C- 15 min
L a b (E)
85.26 4.87 10.14
85.32 6.21 8.93

b)


8°C 10 min
4°C - 15 min
L a b
84.26 5.15 11.83

c)



d)

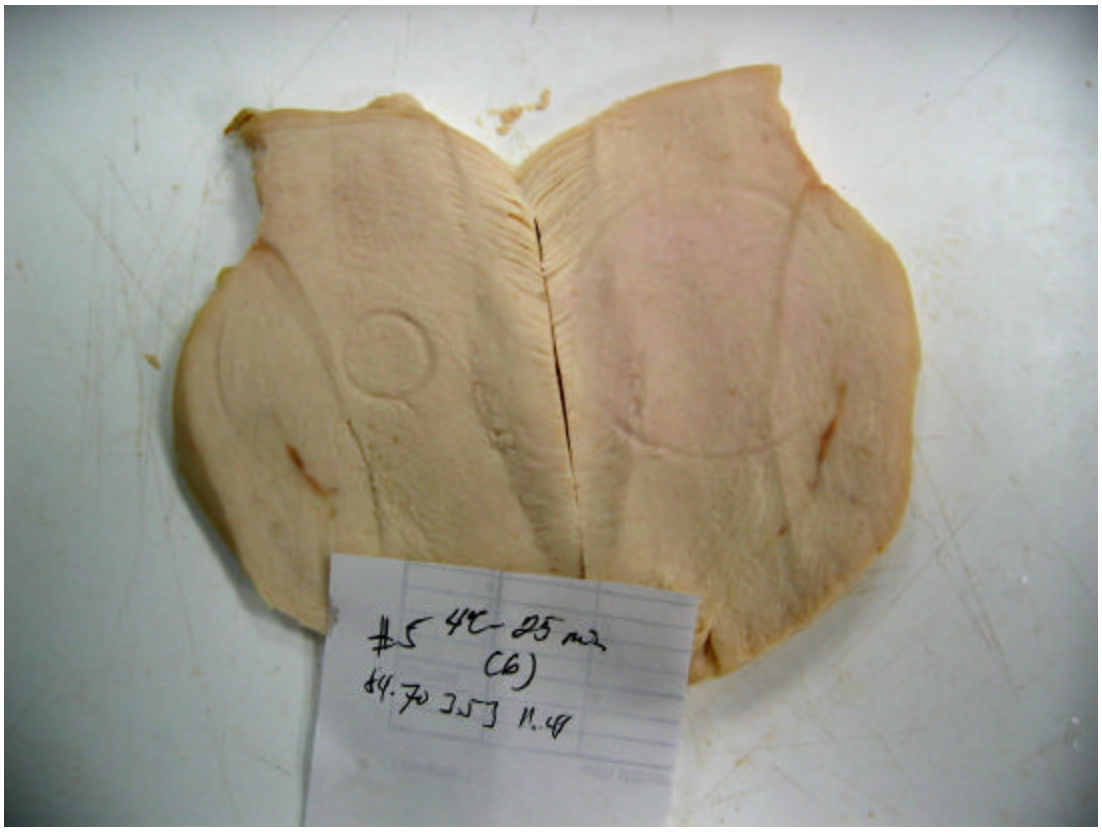


Table 2.3. CIE L*, a* and b* values of raw and cooked marinated chicken breast. Treatment A = 12°C/25 min.

				TREATMENT A					
				RAW					
	Top			Middle			Bottom		
Sample	L*	a*	b*	L*	a*	b*	L*	a*	b*
1	58.88	11.38	14.33				59.86	11.40	15.78
2	61.26	11.78	9.94				62.32	10.90	12.89
3	58.27	10.96	12.41				56.05	9.93	12.23
4	60.24	12.98	12.96				58.15	11.96	12.39
5	58.73	13.12	11.95				56.17	10.54	11.30
DAY 0					COOKED				
1	77.17	6.64	19.04	85.46	5.17	13.76	81.87	4.08	14.89
2	77.83	6.40	16.51	85.01	5.31	10.62	81.96	4.36	12.39
3	78.30	5.48	16.96	83.44	4.92	13.52	81.14	4.08	14.00
4	77.15	6.18	16.03	84.81	5.80	11.49	80.37	4.60	13.62
5	76.05	6.36	15.43	82.58	6.10	11.63	79.39	5.01	121.72
DAY 1									
1	78.46	6.53	16.07	84.69	5.12	13.85	82.27	3.99	14.28
2	78.29	6.52	14.49	84.09	4.69	10.93	81.40	4.60	12.21
3	79.17	4.86	14.71	83.05	4.73	14.02	81.02	3.99	13.67
4	78.44	6.37	13.68	83.59	5.33	11.88	80.02	4.77	13.20
5	76.71	6.35	12.96	82.45	5.69	12.68	78.80	5.01	14.24
DAY 3									
1	78.09	6.71	16.74	81.12	3.98	14.96	84.70	4.88	13.95
2	77.53	6.62	14.50	80.90	4.64	12.56	83.56	4.56	11.37
3	78.85	5.83	14.60	79.33	4.70	13.71	82.52	4.37	14.27
4	78.50	5.98	13.57	79.34	4.50	13.49	83.27	4.90	12.31
5	78.09	5.65	13.19	76.85	4.79	13.76	82.12	5.06	12.70
DAY 5									
1	80.15	4.99	14.12	81.68	3.45	14.48	84.10	4.50	14.30
2	78.42	5.27	14.14	81.23	4.22	12.77	83.69	4.28	11.92
3	78.44	5.84	14.53	79.52	4.71	13.69	83.15	4.11	14.48
4	77.94	6.33	14.30	78.73	3.49	12.96	82.17	4.89	12.86
5	77.22	5.25	14.14	77.91	5.91	13.25	81.59	5.38	12.94
DAY 5				REDUCING AGENT ADDED					
1									
2									
3				NO DATA					
4									
5									

Table 2.4. CIE L*, a* and b* values of raw and cooked marinated chicken breast. Treatment B = 12°C/10min. + 4°C/15min.

	TREATMENT B								
	RAW								
	Top			Middle			Bottom		
Sample	L*	a*	b*	L*	a*	b*	L*	a*	b*
1	60.18	10.62	13.66				59.07	9.94	14.37
2	59.13	9.35	10.12				60.02	7.69	10.54
3	63.08	10.87	14.54				60.57	7.51	14.19
4	60.14	11.59	9.36				61.04	10.05	12.14
5	62.20	11.31	12.63				60.06	10.21	12.47
DAY 0									
1	80.39	4.14	16.78	86.66	4.03	14.24	81.24	3.91	13.92
2	78.30	5.48	16.96	83.44	4.92	13.52	81.14	4.08	14.00
3	79.98	5.09	16.53	86.67	3.24	13.05	82.71	3.93	14.71
4	75.72	8.42	16.50	83.43	6.20	10.70	82.51	5.25	11.98
5	78.79	5.25	16.18	84.08	5.71	13.18	81.44	4.50	12.98
DAY 1									
1	79.65	5.37	15.44	81.33	4.29	13.98	83.89	3.67	14.73
2	78.48	5.41	12.19	82.10	3.62	12.51	83.35	3.43	12.04
3	80.71	4.79	13.91	82.57	4.00	14.06	85.78	3.42	13.24
4	77.78	7.38	13.14	82.10	5.55	11.54	82.43	5.85	11.38
5	79.06	6.27	14.03	81.10	4.54	12.71	83.30	4.99	13.15
DAY 3									
1	78.16	5.93	15.28	81.11	3.34	14.93	83.80	3.45	14.85
2	78.77	5.23	12.12	81.81	3.43	12.57	83.27	3.23	12.37
3	71.40	4.81	14.40	81.95	4.00	14.21	85.72	2.84	13.37
4	77.41	7.04	13.42	81.37	5.17	11.72	81.93	5.66	11.91
5	78.23	6.70	14.09	79.81	4.89	12.60	83.84	4.47	13.88
DAY 5									
1	80.21	4.67	14.76	80.93	3.98	14.12	84.00	4.03	14.80
2	78.26	5.11	12.91	81.94	3.12	12.65	82.90	3.18	12.53
3	80.75	4.82	14.28	80.68	3.94	14.48	85.13	2.76	13.70
4	77.62	6.38	13.79	81.89	5.52	12.20	76.80	8.64	15.19
5	78.24	6.59	14.07	79.60	4.94	12.76	84.61	4.19	14.94
DAY 5	REDUCING AGENT ADDED								
1									
2									
3	NO DATA								
4									
5									

Table 2.5. CIE L*, a* and b* values of raw and cooked marinated chicken breast. Treatment C = 8°C/10min. + 4°C/15min.

	TREATMENT C								
	RAW								
	Top			Middle			Bottom		
Sample	L*	a*	b*	L*	a*	b*	L*	a*	b*
1	57.21	15.72	8.64				57.59	15.00	10.45
2	61.72	13.26	13.66				61.34	13.79	14.35
3	62.25	13.83	8.33				61.44	12.79	9.27
4	59.78	13.04	8.55				59.96	11.38	10.37
5	61.30	11.16	13.52				59.27	11.80	15.02
DAY 0				COOKED					
1	75.92	6.09	13.25	82.75	6.74	10.01	81.48	4.57	11.15
2	79.33	6.20	15.30	85.84	6.24	11.89	82.68	4.44	12.75
3	80.17	4.54	11.33	85.73	5.24	9.59	82.20	4.70	11.06
4	76.33	7.90	16.02	84.40	5.37	10.21	82.39	4.26	12.73
5	77.81	4.97	18.39	85.44	4.17	13.37	82.42	3.73	14.88
DAY 1									
1	76.96	5.98	11.38	80.93	6.74	10.60	80.14	5.04	11.18
2	78.88	6.69	13.31	84.44	5.89	12.74	80.68	4.85	12.37
3	80.31	4.98	11.33	83.59	5.42	10.60	82.20	4.39	11.26
4	76.91	7.84	14.00	83.52	4.88	11.16	80.26	3.93	12.38
5	77.85	5.33	15.51	83.61	3.91	13.90	80.87	3.88	13.04
DAY 3			DAY 3						
1	76.08	7.11	11.70	81.72	6.34	10.89	79.92	5.58	11.31
2	78.97	6.66	13.27	83.72	5.93	13.04	81.01	5.05	12.55
3	80.56	5.15	10.64	84.23	4.83	10.40	81.18	4.41	11.34
4	77.22	7.42	13.46	83.59	4.98	11.31	79.82	3.59	12.66
5	77.96	5.21	16.12	84.45	2.98	14.42	81.34	3.27	14.49
DAY 5			DAY 5						
1	76.58	6.66	11.82	82.37	5.89	11.76	80.17	5.56	11.55
2	79.99	6.59	13.35	84.59	4.01	13.27	80.39	5.27	12.65
3	80.69	4.87	10.81	84.82	4.92	10.62	80.75	3.87	11.67
4	77.41	7.23	13.78	83.94	4.99	11.60	79.98	3.10	12.66
5	78.45	5.37	16.67	84.60	3.14	14.52	80.33	3.80	13.88
DAY 5				REDUCING AGENT ADDED					
1				85.34	7.05	9.90			
2				84.92	7.28	11.01			
3				85.32	6.21	8.03			
4				84.47	6.41	9.19			
5				85.77	4.22	12.74			

Table 2.6. CIE L*, a* and b* values of raw and cooked marinated chicken breast. Treatment D = 8°C/25min.

				TREATMENT D					
				RAW					
		Top			Middle			Bottom	
Sample	L*	a*	b*	L*	a*	b*	L*	a*	b*
1	59.16	13.20	8.75				58.01	13.98	11.38
2	58.53	12.71	11.26				59.69	13.26	13.92
3	61.20	12.15	10.53				59.86	13.37	11.81
4	58.30	12.95	12.11				58.19	12.57	13.00
5	59.67	13.10	14.21				60.03	12.55	17.02
DAY 0				COOKED					
1	78.53	5.79	13.18	85.07	5.45	10.47	80.45	4.66	13.39
2	79.12	4.97	14.79	84.30	4.97	11.61	82.44	3.90	13.00
3	76.72	7.18	14.54	83.89	5.23	11.03	82.48	4.16	11.46
4	78.38	5.80	17.76	84.50	5.51	13.16	81.38	3.53	13.55
5	78.37	5.00	17.78	85.30	4.86	13.23	81.36	4.10	14.90
DAY 1									
1	78.48	6.19	11.77	84.13	5.29	11.27	79.66	4.59	13.07
2	79.29	5.18	13.38	83.67	5.21	12.41	81.02	4.07	13.37
3	76.43	7.53	13.08	83.23	5.56	12.01	81.07	4.61	11.69
4	77.61	7.24	17.05	83.47	5.59	13.88	80.36	4.23	13.26
5	77.77	6.56	16.64	84.32	4.83	13.86	80.67	4.15	13.52
DAY 3									
1	77.91	6.74	11.93	84.62	5.13	11.23	81.02	4.42	12.11
2	79.18	5.62	13.26	83.38	4.91	12.56	81.52		13.06
3	76.14	7.56	13.63	82.89	4.70	12.01	79.51	4.87	12.13
4	77.74	7.13	16.13	83.26	5.44	13.94	80.25	4.16	13.11
5	78.38	6.03	15.80	84.17	4.00	13.90	81.94	3.88	13.64
DAY 5									
1	81.51	3.41	11.80	84.00	5.24	11.47	79.48	4.22	12.81
2	78.92	5.61	13.02	83.04	4.58	12.83	81.07	3.87	14.80
3	77.07	6.77	12.68	83.01	5.12	12.65	80.12	4.68	12.26
4	77.13	6.60	16.09	84.69	4.45	11.56	81.04	4.18	13.42
5	78.29	6.90	16.15	83.15	4.80	14.14	80.79	4.05	13.44
DAY 5				REDUCING AGENT ADDED					
1				84.55	5.83	9.39			
2				84.00	5.32	10.88			
3				82.34	5.59	10.31			
4				83.24	5.72	12.42			
5				84.77	5.29	12.01			

Table 2.7. CIE L*, a* and b* values of raw and cooked marinated chicken breast. Treatment E = 4°C/25min.

	TREATMENT E								
	RAW								
	Top			Middle			Bottom		
Sample	L*	a*	B*	L*	a*	b*	L*	a*	B*
1	63.25	11.79	7.42				60.47	18.52	9.49
2	62.08	11.31	13.81				61.07	12.40	14.29
3	58.15	12.97	11.49				57.17	12.27	13.29
4	62.25	12.99	8.61				61.16	12.78	12.53
5	60.18	12.62	7.20				58.70	13.48	11.08
DAY 0				COOKED					
1	81.00	4.54	11.83	85.78	5.14	8.27	80.62	4.25	10.98
2	81.40	4.13	14.94	84.61	3.68	11.11	83.05	3.73	12.34
3	75.55	6.59	14.24	81.11	5.62	13.54	80.94	4.29	12.44
4	79.11	5.81	13.40	84.74	4.63	9.56	82.16	4.42	11.36
5	76.96	5.16	13.46	84.24	5.06	10.46	82.58	3.71	11.93
DAY 1									
1	81.07	5.45	10.95	85.65	5.02	9.10	81.99	4.31	11.24
2	81.99	4.65	13.12	84.77	4.06	11.75	82.83	4.15	12.19
3	76.97	6.68	12.25	80.30	4.38	14.01	79.77	5.12	12.84
4	80.02	5.82	11.14	84.79	5.07	10.25	81.39	4.24	11.10
5	78.26	5.61	12.04	84.06	5.04	11.39	82.75	3.81	11.50
DAY 3									
1	81.54	5.51	10.99	84.90	4.94	9.16	80.35	4.36	11.56
2	79.67	5.24	14.55	85.81	3.37	12.32	80.34	4.64	12.29
3	77.73	5.67	12.65	81.03	4.17	15.36	78.96	5.56	12.94
4	78.66	5.66	12.96	84.83	5.19	10.34	81.07	4.95	11.33
5	77.70	5.34	12.15	83.09	5.26	11.46	81.42	3.78	11.52
DAY 5									
1	81.43	5.40	11.48	85.81	5.03	9.20	80.77	3.91	11.65
2	82.14	4.30	13.09	84.99	3.36	12.63	82.36	4.34	12.55
3	77.31	6.43	13.42	79.96	4.44	15.30	78.28	5.69	13.65
4	80.40	5.81	11.78	84.94	4.74	10.65	81.81	4.29	11.44
5									
DAY 5				REDUCING AGENT ADDED					
1				85.42	5.65	7.18			
2				84.42	4.36	10.19			
3				79.53	5.58	8.08			
4				84.73	5.56	12.58			
5									

Table 2.8. pH and ORP values of meat samples measured at different days. Treatment codes are the same as in the previous tables.

	RAW									
	Treatment A		Treatment B		Treatment C		Treatment D		Treatment E	
Sample	pH	ORP	pH	ORP	pH	ORP	pH	ORP	pH	ORP
1	5.60		5.82		5.97		5.80		5.83	
2	5.64	NO	5.89	NO	5.51	NO	5.82	NO	5.73	NO
3	5.93	DATA	5.63	DATA	5.68	DATA	5.76	DATA	5.69	DATA
4	5.75		5.75		5.77		5.70		5.70	
5	5.66		5.66		5.70		5.67		5.80	
After 24 hrs.					RAW MARINATED					
1	5.89	174.2	5.88	218.0	5.92	162.6	5.93	222.9	5.76	208.2
2	5.95	203.3	5.86	214.0	5.58	220.9	5.88	212.8	5.77	202.7
3	5.97	201.9	5.83	215.5	5.86	217.5	5.80	213.6	5.62	200.8
4	5.92	201.1	5.93	214.4	5.82	231.6	5.71	203.1	5.71	193.5
5	5.75	183.9	5.82	209.5	5.79	210.1	5.73	243.9	5.78	188.4
DAY 0					COOKED					
1	5.98	151.6	6.01	129.7	6.05	167.9	6.01	167.9	6.07	171.1
2	5.98	171.9	6.06	143.2	6.10	125.2	6.20	125.9	6.21	164.0
3	6.02	140.6	6.08	137.1	5.96	124.0	6.10	124.0	6.05	192.8
4	6.00	144.2	6.00	151.1	6.01	138.8	5.80	138.8	5.97	119.1
5	5.86	131.8	6.05	139.1	5.89	140.0	5.85	140.0	5.97	106.0
DAY 1										
1	6.06	179.4	6.03	206.7	6.26	216.4	6.21	172.7	6.21	216.8
2	6.13	264.4	6.08	214.3	6.07	213.9	6.20	186.7	6.14	233.2
3	6.24	243.1	6.10	207.2	6.14	220.6	6.11	211.5	5.99	223.0
4	6.13	212.0	6.15	211.4	6.21	165.1	6.22	205.2	6.08	232.4
5	6.01	228.5	6.08	215.0	6.13	215.7	6.05	210.4	6.13	224.7
DAY 3										
1	6.08	232.7	6.05	221.4	6.16	251.0	6.10	209.0	6.26	252.8
2	6.09	236.7	6.18	240.2	6.07	260.6	6.21	216.2	6.31	235.7
3	6.22	227.9	6.12	212.1	6.16	230.0	6.12	207.4	6.15	234.9
4	6.11	228.9	6.11	246.6	6.15	200.3	6.16	229.6	6.15	238.2
5	6.00	235.7	6.07	224.3	6.10	252.8	6.14	228.0	6.13	241.0
DAY 5										
1	6.17	284.3	6.14	336.0	6.28	245.6	6.17	192.3	6.09	213.4
2	6.21	283.0	6.19	357.2	6.04	246.1	6.19	180.7	6.18	218.3
3	6.24	277.9	6.18	305.9	6.18	105.7	6.17	227.1	6.09	210.1
4	6.06	255.1	6.12	295.6	6.21	203.2	6.19	208.3	6.06	193.6
5	6.05	274.9	6.11	287.0	6.10	239.3	6.10	216.7	6.06	199.1

Table 2.9. ORP value of cooked chicken breast meat after addition of sodium dithionite. Treatment codes are the same as in the previous tables.

Day 5	Treatment C	Treatment D	Treatment E
1	-251.0	-127.6	-190.0
2	-191.0	-80.0	-200.0
3	-194.4	-231	-218.0
4	-180.3	-233.0	-191.0
5	-190.0	-169.0	-193.0

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CHAPTER 3

CONSTANT AND STEPWISE STAGED TEMPERATURE VACUUM MARINATION OF MEATS: EFFECTS ON MARINADE ABSORPTION AND RETENTION[1]

[1]Palang EY, Toledo RT. 2004. To be submitted to the Journal of Food Science

ABSTRACT

Marination by vacuum tumbling is commonly used in the meat industry, however, with very few exceptions, an isothermal process is used. This study evaluated effects of temperature on the process. Boneless chicken breasts were vacuum tumbled isothermally at different temperatures or by a two stage stepwise temperature process from low to high temperature or vice versa. All other conditions such as rotational speed, vacuum, and tumbling time were held constant. Responses evaluated were per cent marinade pick up, cooking yield, and free water content as per cent expressible moisture.

Values of the response variables were dependent on marination temperature. Highest marinade pick up was observed in an isothermal process at 12°C. However, cook yield was highest when meat was vacuum tumbled at 12^{o}C for 10 min followed by 15 min at 4^{o}C. The per cent expressible moisture was highest when marination temperature was 8^{o}C for 25 min. A significant negative correlation was found between temperature and per cent expressible moisture ($r = -0.530$, $P<0.05$), and per cent marinade pick up ($r=-0.405$, $P<0.05$). The temperature during a vacuum tumbling process can significantly affect marinade absorption, cook yield and expressible moisture, thus to obtain a consistent marinated product, temperature of meat and marinade and heating or cooling capability on the equipment should be considered.

INTRODUCTION

Long before its use in commercial meat processing operations, marination technology had been utilized in domestic meat cookery. Webster's dictionary defines "marinade" as a pickle, and marination implies pickling or soaking meat in a marinade solution for hours. This was commonly done in domestic kitchens, but to accelerate marinade absorption by meat, commercial marinades are injected, tumbled or massaged using mechanical systems. Marination technology has expanded from poultry meats to red meats and fisheries products. Furthermore, the objective of marination has expanded from flavoring meats to tenderization and improvement of cooked meat juiciness.

Indices of successful marination consists of: complete absorption by meat of all added marinade, avoidance of liquid separation from the raw meat (purge) and the retention of marinade on cooking to maximize cooked yield. Meats with high cooked yields are generally tender and juicy. These desirable characteristics of a successfully marinated meat are attained by the interaction of marinade ingredients with the meat matrix. It is generally assumed that salt soluble proteins are extracted, actomyosin is broken down to actin and myosin, and functional ingredients such as phosphate opens up spaces within the protein matrix to trap water. Marinade ingredient functionality is maximized only when the marinade is uniformly incorporated into the meat. Otherwise, marinade ingredient and meat interaction will be localized if the marinade is only found at or near the surface of the meat. In addition, marinade should be well distributed in the meat as mechanical action such as tumbling or massaging is applied to promote the functional ingredient interaction.

The conventional method of marinating meat is an isothermal operation at temperatures from near the freezing point to 4^{o}C. Marination at low temperature is practiced because of the

convenience of working with raw materials already at low temperatures designed to inhibit microbial growth. To accelerate marinade absorption by meat, vacuum tumblers and massagers are used in the industry. Once the meat and marinade are mixed, most mechanical marination systems do not provide additional heating or cooling. Some systems have external jackets through which cold or heated transfer fluid may be circulated thus providing a means of temperature control.

At room temperature, meat muscle bundles are more flexible and easier for marinade to penetrate since marinade viscosity and surface tension is lower. However, solubilization of salt soluble proteins in raw muscle is better at lower temperature. Under this premise, a staged temperature marination profile could increase the efficiency of marinade delivery into the meat.

In this study, we will investigate the effects of different marination temperature schedules in either an isothermal process or a staged temperature process using different time of tumbling at the designed process temperature. Responses evaluated are marinade uptake by the raw meat, cook yield, and expressible moisture in the cooked product.

MATERIALS AND METHOD

Marination

Two vacuum tumblers were used in the experiment a 50 pound capacity vacuum tumbler UMEC model 1102 (UMEC, City of Industry, CA), and Pollar Massager model 1120 (Wolf-tec Inc., Kingston, NY) were used. The latter was used consistently on the 4^{o}C part of the stepwise stage vacuum marination to confound the effect of the vacuum tumblers in the experimental design (Split-splot experimental design). Both tumblers were used for the 4^{o}C vacuum tumbling marination. A high capacity vacuum pump (Soge vac model UV 25, Leybold Vacuum, Export PA) was used to draw the vacuum.

Deboned split chicken breasts were obtained from a local processing plant. Each batch used in the tests weighed from 1.545 -1.885 kg. Several pieces of chicken breast were assigned to a batch, randomly. Each batch was used for one treatment. On a single day, four treatments were conducted, except for the day when the control treatment was carried out. The experiment consisted of two levels of temperature and four levels of marination time. Including the control, there were a total of 9 experimental treatments. Control was tumble marinated in a vacuum at 4°C for 25 min. Raw chicken not used in one day was stored in a 4°C walk-in cooler for the next day experiments.

The marinade was prepared to obtain the target concentrations of functional ingredients when absorbed by the meat at 20 per cent of meat weight. Target concentration in the marinated meat was 1.0% NaCl and 0.35% sodium tripolyphosphate (STPP). The functional ingredients were dissolved in deionized water. Citric acid was added to adjust marinade pH to 6.0. This marinade pH and the amount of marinade added have been determined in preliminary experiments to raise the marinated meat pH to 5.9 from 5.7 in the untreated chicken meat. The tumbler speed was set at 10 rpm and tumbling was started after the vacuum gage registered 25 inches Hg (130 mBar absolute).

To identify the treatments, the control was designated Treatment 0, while the test treatments were: Treatment (1) - 8°C for 5 min. and 4 °C for 20 min.; Treatment (2) – 8°C for 10 min. and 4°C for 15 min.; Treatment (3) – 8°C for 15 min. and 4°C for 10 min.; Treatment (4) – 8°C for 25 min.; Treatment (5) – 12°C for 5 min. and 4°C for 20 min.; Treatment (6) – 12°C for 10 min. and 4°C for 15 min.; Treatment (7) – 12°C for 15 min. and 4°C for 10 min.; Treatment (8) – 12°C for 25 min.

The pH of individual chicken breast pieces was measured prior to marination. Five pieces of chicken meat were placed inside Cryovac barrier bags along with the marinade. A type-T thermocouple was inserted into the geometric center of the chicken meat and the bag was heat sealed in a vacuum packaging machine. The packaging machine (Henkelman 600) vacuum was set to 2 inches vacuum before heat sealing. The thermocouple measured the temperature as the bag with the chicken meat was tempered to the desired temperature. Temperature adjustment was done by either exposing the packaged meat to air at the target temperature or by immersing packaged meat in a water-ice slush mixture. Once the target temperature was reached, the meat was removed and placed inside the vacuum tumbler.

After marination at the first temperature was complete, the meat was removed from the tumbler and exposed to a water-ice slush mixture until the proper temperature was reached. The package was then transferred to a second tumbler. There were two tumblers used in the experiment. One tumbler was left overnight at 4^{o}C and the second tumbler was left in either 8 or 12^{o}C walk-in cooler or temperature controlled room depending on the desired treatment temperature.

Marinade Absorption

After the meat was marinated, the vacuum in the bag was released by punching a hole in the bag, and then the meat was stored 24 h in the same bag at atmospheric pressure in a 4^{o}C walk-in cooler. The meat was then removed from the plastic bag, blotted with paper towels, and weighed. The unabsorbed marinade was discarded. Percent marinade absorption was calculated as follows:

$$\% \text{ absorbed} = \frac{(\text{wt. 24 h after marination} - \text{unmarinated weight})}{\text{unmarinated weight}} \times 100$$

Percent Cook Yield

After recording the per cent marinade absorption at each treatment, the meat pH was measured, and then the meat was transferred to baking pans for oven cooking. Meat was cooked in a domestic kitchen oven (GE model JBP90) set to 375 ^{o}C and a type-T thermocouple was inserted into the geometric center of the chicken breast. Once the internal temperature reached 72^{o}C, the baking pan was immediately taken out of the oven and the meat was allowed to cool down exposed to ambient air at room temperature. The pH of the cooked meat was measured after meat had cooled down to room temperature, and weight was measured. The per cent cook yield was:

$$\% \text{ Cook Yield} = \frac{(\text{wt. after cooking} - \text{green wt.})}{\text{green weight}} \times 100$$

Percent Expressible Moisture

Percent expressible moisture was not measured on the same day of cooking. The meat was placed in a reclosable polyethylene bag and stored in a 4^{o}C walk-in cooler until used for the determination of per cent expressible moisture.

Percent expressible moisture was determined by cutting three 2.1 cm cubes from each cooked whole breast fillet. Samples were taken from three chicken breast fillets from each treatment. Nine separate measurements were made from each treatment. Each cube of cooked meat sample was weighed before and after compression using an Instron Universal Testing Machine. The measurement was done at a cross-head speed of 50 mm/min and compression was done until the applied force was 400 N. The load was applied for 1 min before the cross-head was lifted to release the applied force. Sheets of filter paper were placed both under and above

the sample to absorb moisture released during compression. The weight of filter paper with the released moisture was measured. The percent expressible moisture was determined as follows:

$$\% \text{ expressible moisture} = \frac{(\text{wt. filter paper before compression} - \text{wt. after compression})}{\text{wt. filter paper before compression}} \times 100$$

Statistical Analyses

The experimental design for marination was a split-splot experimental design while for the percent cook yield, percent marinade absorption and percent expressible moisture was a complete randomized block design. SAS software (SAS Institute, 1989) GLM procedure and option LSD was used to determine the statistical differences in percent cook yield, percent marinade absorption and percent expressible moisture at a=0.05. The experiments were replicated twice.

RESULTS AND DISCUSSION

Meat pH

The mean pH of the raw chicken breasts in each treatment ranged from 5.725 to 5.913 (Table 3.1). The pH of raw meat in Treatment 1 was statistically different from the rest of the treatments. However, after marination the meat pH equilibrated to near pH 5.9. The pH of individual pH among samples in the treatments was found to be not significant ($P<0.05$) (Table 3. 1).

If there is a big difference in meat pH among treatments, results of the experiment can be biased because pH affects water binding properties of meat proteins. In addition, interaction of marinade functional ingredients with meat can also be affected by pH. The marinated meat pH must not be significantly different between treatments to isolate the temperature effects from the pH effects. The buffering capacity of the marinade to change the raw meat pH to the desired

level after marination is important in the process. The marinades used in these experiments demonstrated their capacity to adjust meat pH to the desired value. The selected target marinated meat pH of 5.9 was based on first study, which showed that at this pH, a lower incidence of pinking and good uptake of marinade was obtained. The pH of marinated meat before cooking was shown to be essential in maximizing marinade absorption, reduction of incidence of pinking, and pH affected the thermal stability of myoglobin against denaturation and changed the ORP in the marinated meat.

After cooking, the pH of cooked chicken meat increased above the raw values in all treatments. Treatment (3) showed the highest increase in pH of about 0.2 pH units above the raw values while treatment 2 showed the least increase of 0.066 units. An average pH increase of 0.15 was observed in all treatments. The pH difference different treatments could be due to differences in penetration of marinade into the meat and the interaction of functional ingredients with meat proteins. Degree of meat protein solubilization and protein degradation during marination and cooking releases free amino acids which themselves have good buffering capacity. Mean pH of cooked meats in the different treatments ranged from 5.99 to 6.13. The pH of marinated meat were not significantly different ($P<0.05$) among treatments, however, after cooking, pH of Treatments 1, 2, 4, 6 were significantly different from Treatments 3, 5, 7 and 8 (Table 3.1).

Marinade Absorption

Some treatments did not absorb all the added marinades as evidenced by the presence of a pool of liquid with the meat in some treatments after marination. At 24 h post-marination, all treatments were observed to release marinade at varying degrees (Table 3.2). Average marinade absorption was not significantly different among the treatments, although meats marinated at

12°C first appear to absorb the most marinade. Our results was similarly reported by Proctor and Cunningham (1987), that marinade absorption was higher in broiler drumsticks marinated at 23°C compared to 4°C. Treatments 6, 7, and 8 which were marinated at 12°C for various times followed by reduced temperature for the remainder of the 25min. total treatment time showed increasing absorption with increasing time at 12°C. There is a trend of increasing marinade absorption with increasing temperature on the first step of a staged process. For processes carried out at a single temperature for the full 25 min, the trend is for marinade absorption to increase with increasing temperature 15.8%, 16.3%, and 17.2% at 4, 8 and 12°C, respectively. Increased marinade absorption at higher temperature was expected since diffusivity of marinade functional ingredients increases with increasing temperature. In addition, the stiffness of the meat decreases with increasing temperature promoting more flexing of the meat as the meat is tumbled at higher temperature.

The raw marinade retention is important to processors who sell packaged raw product. The pack-off weight is the basis for the net weight label and consequently what the processor is paid for the product so maximizing the raw marinated weight maximizes processor revenue. Unabsorbed marinades usually separate out in the holding bin for the marinated product and are discarded after all the meat pieces are packed. On the other hand, loosely held marinade in the meat will separate during storage and small amounts can be absorbed by the absorbent pad in the package but excessive marinade separation to the point where liquid is present in the package could give consumers the perception that water is being sold to them for the price of meat.

Cook Yield

The yields of cooked marinated chicken breast meat are shown in Table 3.2. While no significant differences were found with the marinade absorption among different treatments,

mean cook yield values separated into three groups with Treatment 6 (12^{o}C/10 min + 4^{o}C/15min.) different from all the others and a highest yield of 88.9%. This indicates that the high temperature step in the process must be for enough time to promote diffusion of functional ingredients followed by a low temperature step to maximize protein-functional ingredient interaction. There was no significant correlation between values for cook yield and marinade absorption.

Cook yield of product from single temperature treatments were lower than in the staged treatments and the trend was higher yield with increasing temperature (Treatments 0, 4, and 8). However, staged temperature treatments did not follow a pattern with temperature as interactions between the first and second temperature treatment and treatment time at either temperature resulted in some combinations doing better than others. Treatments 2 and 3 and 7 were the second highest yield grouping and they involved tumbling for a short time at 8 or 12 °C and the majority of the tumbling time was at 4 °C. Cook yield had significant positive correlation ($r = 0.449$, $P<0.05$) with temperature and a negative correlation ($r = -0.455$, $P<0.05$) with time of marination at the elevated temperature in a staged process. An optimum marination time was found to be at 10 min. at 12^{o}C followed by 15min. at 4°C (Treatment 6).

Meat with high absorbed marinades does not guarantee maximum cook yield. Achievement of high cook yield could be attributed to several factors. One factor is the site of retention of water in the meat. Water may be in areas where water movement is restrained by surrounding structures. Water found in extracellular spaces could easily flow out of the meat or be squeezed out of the meat as muscle fibers contract on heating. Water bound by native protein can be released as the protein is heat denatured. Furthermore, the gel matrix of heat-set salt soluble proteins may be broken down with excessive heating or high temperature gradients

(Schmidt 1984). Water retention in meat is not a simple mechanism. Obviously, marinade has to be successfully delivered first into the meat system. Marinade must penetrate different areas in the meat before it can be retained. On cooking, all free water that entered as marinade and water released by protein denaturation must remain in the muscle by some form of entrapment. The denatured protein gel network can trap some of the water if protein-protein interaction is moderate leaving spaces for water to be trapped. Water in sites in the muscle close to the surface can easily exude out of the meat, therefore adequate penetration of marinade is necessary to maximize yield.

During cooking, the meat contracts which forces the marinade out of the meat. Free water or water that is not strongly bound to meat is released. Water released during meat cooking can possibly be from the water bound to the meat by physical force or those in the extracellular spaces. Conversely, the water retained may be due to the entrapment by the protein gel matrix, those chemically bound to the protein, and those remaining in the interfibrillar space. Heat mediated contraction of the muscle fibers and collagen will eventually force out unbound marinade while bound water is retained.

Solubilization of the salt soluble proteins also plays a major role in the retention of water. Salt soluble protein acts as a binding agent and retain water in meat (Babji et al. 1982). Aside from the electrostatic repulsive force of Cl^- ions attached to the filaments, salt depolymerises myosin molecules and other components that plays a significant role in absorption/retention of marinade (Offer et al. 1983). Since protein plays a significant role in water retention in the meat, it is important for the protein to be available to either absorb or entrap the water (Xiong and Brekke 1989).

The mechanism of marinade absorption should be different from that of cook yield. Raw protein generally holds more water than denatured protein and there are enough open spaces within the raw muscle to trap water. Thus, marinade absorption generally does not vary very much between treatments.

The role of the site occupied by water in marinated meat is still not clearly elucidated. Some sites may hold the water stronger than other sites. Elucidation of the location of these binding sites will aid greatly in designing marination processes which will induce the maximum retention of marinade in the meat during raw storage or during cooking. The proportion of water entrapped by the gelled myosin extract relative to total marinade retained has still to be determined.

Expressible Moisture

Expressible moisture is free water that is easily pressed out of the meat by compression. A high expressible fluid value is an indication that a greater proportion of the water present is loosely held in the meat. A high expressible fluid value in cooked meats may mean high sensory juiciness perception of the cooked product and could be a desirable attribute. Treatment 4 yielded the highest value of expressible moisture (Table 3.2) indicating that the tumbling process carried out totally at 8°C did not induce good binding of water to the meat components. The results showed that temperature and time of marination had different effects on expressible moisture.

Temperature was found have an opposite effect on expressible moisture i.e. marinating at higher temperature yields lower expressible moisture content. A significant negative correlation ($r = -0.530$, $P<0.05$) was found between temperature and %expressible moisture. Furthermore, a negative correlation ($r = -0.405$, $P<0.05$) was found between marinade absorption and

expressible moisture which means that water was tightly held or bound in treatments with high marinade absorption values. No significant correlation was found between the cook yield and expressible moisture.

The negative correlation between marinade absorption and expressible moisture could possibly be due to differences in locations of water in the meat. At high marinade absorption, water may have penetrated the interfilament spaces within the myofibrils. Thus, the expressible water could come from the extracellular spaces, and from the spaces between the myofibrils. According to Offer et al. (1983), water is located in the meat interfilament spaces, extracellular and intermyofibrillar spaces with a negligible presence of water bound to protein molecules. Marinade absorption was measured after 24 hours of meat storage therefore the values are a measure of relatively stronger-bound marinade. Marinade that purged out of the meat could have come from the extracellular and intermyofibrillar spaces. The water left after cooking are the water that were tightly bound to the meat and were only expressible upon an application of external force. However, this still have to be elucidated as to which site of water plays a significant role on a marinated meat with high marinade absorption.

Optimum Tumbling Condition

The definition of an optimized marination depends on which among the three dependent variables (marinade absorption, cook yield, and expressible moisture) will be considered most important in setting the desired product attributes. Or, if all of the dependent response variables are important, in this case, the treatment with the highest sum of cook yield, expressible moisture and marinade absorption could be considered the optimum condition for marinating poultry meat.

Besides the three dependent response variables (marinade absorption, cook yield and expressible moisture), there are other factors that need to be considered in deciding which combination of temperature and time is optimum for marination. Economic and practical approaches to the processing of poultry meat have to be considered. Raising the meat temperature say from 4 to 12^{o}C may seem economically unfeasible due to the amount of energy to be used in order to raise the temperature of the meat. Furthermore, microbiological issues may arise as a result of an increased temperature. However, if after processing the raw meat temperature is already or close to 8 or 12^{o}C , it will be practical to choose the temperature and time combination to process the meat in order to give a higher value of either marinade absorption, cook yield, and expressible moisture or considering the sum up value of the three variables. Cooling down the temperature eventually to 4^{o}C is favored since meat temperature is kept at refrigerated temperature when it leaves the processing line.

CONCLUSION

In conclusion, the determination of optimum marination condition is subject to the intended use of the marinated product by the processor whether marketed raw or cooked. The response variables of marinade absorption, cook yield and expressible moisture do not respond similarly to temperature and time of marination. Furthermore, the chicken meat temperature condition has to be considered when choosing the temperature of marination since increasing or lowering the meat temperature could have economic liabilities. However, looking at the results individually the following are recommended: marinade absorption – Treatment 8 ; expressible moisture – Treatment 4; cook yield – Treatment 6.

It is evident in this experiment that temperature of marination is important to consider since this can result in significant inconsistencies or variability in marination process,

particularly involving %cook yield and %expressible moisture. Furthermore, at the practical stand point, marinade absorption could be affected as well. This is due to the fact that time and temperature of marination have an impact on the delivery, functionality of the marinade, and interaction between meat and the marinade.

Table 3.1 Percent marinade absorption (%MA), percent cook yield (%CY), and percent expressible moisture (%EM) of marinated chicken breast fillet. Treatment codes: 1 = 4°C/25min.; 2 = 8°C/5min. + 4°C/20min.; 3 = 8°C/10min. + 4°C/15min.; 4 = 8°C/15min. + 4°C/20min.; 5 =4°C/25min.; 6 = 12°C/5min. + 4°C/20min.; 7 = 12°C/10min. + 4°C/15min.; 8 = 12°C/15min. + 4°C/10min.; and 12°C/25min. Data in a column with different letter was significantly different (P<0.1) by LSD.

Treatment	%PK	std. dev.	%CK	std. dev.	%EM	std. dev.
1	115.89ab	1.12	85.19bc	0.62	26.25ab	2.45
2	115.30b	1.38	86.11bc	0.16	25.20bc	2.59
3	115.71ab	0.81	87.57ab	0.09	23.18cd	2.65
4	116.73ab	1.43	87.51ab	1.15	22.17de	2.59
5	116.34ab	1.52	84.35c	1.29	27.53a	3.05
6	115.94ab	1.30	86.44abc	0.47	25.36abc	2.53
7	116.17ab	0.78	89.09a	2.72	25.08bc	1.49
8	117.06a	0.20	87.00abc	0.77	20.01e	2.14
9	117.25a	1.28	86.65abc	0.57	21.26de	2.59

Table 3.2. Average meat pH of poultry meat as raw, marinated, and cooked in each treatment. Data in a column with different letters was significantly different (P<0.05) by LSD.

Treatment	Temperature 1	Time 1	Temperature 2	Time 2	pH		
					Raw	Marinated	Cooked
	°C	min	°C	min	Meat	Meat	Meat
0	4	25	4	0	5.885ab	5.888a	6.067ab
1	8	5	4	20	5.913a	5.860a	5.994b
2	8	10	4	15	5.762ab	5.924a	5.990b
3	8	15	4	10	5.874ab	5.946a	6.136ab
4	8	25	4	0	5.828ab	5.865a	5.999b
5	12	5	4	20	5.815ab	5.858a	6.046ab
6	12	10	4	15	5.725b	5.870a	6.024b
7	12	15	4	10	5.767ab	5.886a	6.036ab
8	12	25	4	0	5.751ab	5.842a	5.992b

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CHAPTER 4

MARINADE PENETRATION TRACING BY THE TRIVALENT EUROPIUM ION AND COMPARISON WITH SOLUBILIZED SALT SOLUBLE PROTEIN [1]

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ABSTRACT

Chicken breast meat was marinated using a vacuum tumbler at 4 and 10°C. Salt soluble protein was recovered by slicing the meat both transversely and by sectioning on the bone side. The amount of salt soluble protein extract recovered was determined by the Biuret method. The chicken breast was also cross-sectionally cut for determination of marinade penetration in the meat. The depth of marinade penetration in marinated meat was traced by fluorescing trivalent europium ion. The image was captured by a CCD camera mounted to an inverted microscope. Thenoyltrifluoroacetone(TTA) and trioctylphosphine oxide(TOPO) were used as chelating agents to Eu^{+3}.

The result showed that the Eu-TTA-TOPO complex effectively demonstrated fluorescence in meat matrix and traced marinade penetration in raw chicken breast. Chelating agents TTA and TOPO successfully prevented the interference of phosphate from the marinade from quenching fluorescence from Eu^{+3} in the presence of pH 4.7 acetate buffer. Eu^{+3} penetration in chicken breast meat was found to reach up to about 3.4 and 4.7mm at temperatures 4 and 10°C, respectively. Consequently, the amount of marinade absorbed was found slightly higher at 10°C than in 4°C. In addition, the amount of recovered salt soluble protein was higher initially at the first few millimeters in chicken meat surface at 4°C. At 10°C, recovered salt soluble protein was found to be greater in the deeper part of the meat, in comparison to meat marinated at 4°C.

Marinade penetration in vacuum tumbled meat was different at 4 and 10°C, and this was effectively demonstrated by tracing the marinade in the meat matrix using Eu^{+3}.

INTRODUCTION

Marinade absorption and retention by meat are the primary goals of an industrial marination process. The interaction between absorbed marinade functional ingredients and meat proteins strengthens water binding in the meat and prevents the loss of water when the meat is cooked. The net result is improved sensory juiciness when the product is consumed. One of the more common marination processes used in industry is vacuum tumbling. Marinade is infused into the meat by repeatedly squeezing and relaxing the meat as it is successively lifted and dropped as the tumbler rotates. Tumbling meat and marinade in a vacuum facilitates penetration of marinade into the meat's interior resulting in better marinade absorption and retention compared to tumbling at atmospheric pressure. A basic understanding of marinade distribution in vacuum tumbled marinated meat will be beneficial in formulating marinade and in the selection of optimum processing conditions.

In tumbling marination, marinade surrounds the individual meat pieces therefore functional ingredient penetrates the meat from the surface. Capillary and molecular diffusion are the predominant mechanisms for the ionic and low molecular weight organic compounds in the marinade to penetrate the meat. Very few studies on diffusion of marinade functional ingredients into meat during a vacuum tumbling process are reported in the literature. Most studies traced marinade absorption by using dyes that have molecular weights much higher than the primary marinade ingredient, NaCl. The dyes fluorescein (mol. wt. 376) and FD&C Blue No. 1 (mol. wt. 466) have been used (Xiong and Kupski 1999; Kay 2001). Tracing marinade penetration by using dyes and then either thin slicing or confocal scanning laser electron microscopy (CSLM) methods may not show actual penetration of the low molecular weight marinade ingredients.

Trivalent europium ion (Eu^{+3}), a lanthanide series atomic element member, exhibits strong luminescence in visible range and is highly stable (Arnaud and Georges 2003; Dimitriev and Kisyluk 2003). Its molecular weight of 151 g/gmol is relatively smaller than the dyes used in recent studies. The europium ion, due to its lower molecular weight, may diffuse similarly as marinade ingredients.

The objective of this study was to determine the depth of marinade penetration and salt soluble protein solubilization in interior layers of meat vacuum tumbled with marinade at 4 and 10^{o}C. Trivalent europium ion was used to trace marinade penetration.

MATERIALS AND METHOD

Vacuum-Tumbling

Marinade was prepared with 0.003M $EuCl.6H_20$ (99.9%, Aldrich Chemicals). Other marinade components were 6% NaCl, 2.1% sodiumtripolyphosphate (Brifisol) and the rest was deionized water. After the solutes were dissolved, citric acid was added to adjust marinade pH to 5.9. Boneless chicken breast (95.6 to 115 g individual piece weight) was purchased from a local processing plant.

The chicken breasts were divided into two batches, one each for 4 and 10^{o}C vacuum-tumbling marination for 25 min. The meat samples were first tempered to the temperature desired prior to vacuum tumbling. The tumbler (UMEC model 1102, City of Industry, CA) was operated at 10 rpm and 25 inches Hg vacuum. The vacuum tumbler was placed inside a controlled temperature room. The weight of each batch before and after marination was measured. The marinated chicken breasts were placed over a 9 mm thick 36x36 cm stainless steel slab lined with a sheet of Teflon. Another sheet of Teflon was placed over the meat and another stainless steel slab was placed above the upper Teflon sheet. The stainless steel slabs

and the sheets of Teflon material were pre-cooled overnight in a walk-in freezer at -20 °C. Four 2.5 cm thick x 2.5 cm diameter Teflon discs were placed on the four corners of the stainless steel slabs to limit the deformation of the meat as the upper plate weighed down on the meat. The Teflon sheets were used as release agents to prevent the meat from sticking to the cold stainless steel surfaces.

After about 10 min between the stainless steel slabs, the chicken breasts were recovered and trimmed. Each trimmed piece was then sliced in sections about 1 mm thick using a rotary blade meat slicer (Hobart). The pieces were sectioned across and along the muscle fibers for examination under a digital microscope and for salt soluble protein analysis respectively.

Specimen Preparation and Fluorescence Examination

The Eu^{+3} ion fluorescence procedure was modified based on the information taken from the works of Scaff et al. (1969), and Brennetot and Georges (2000). The strip of marinated chicken breast section was placed on a glass slide and washed with a solution composed of a mixture of 0.005M TOPO (trioctylphosphine oxide, Aldrich Chemicals) and 0.01M TTA (thenoyltrifluoroacetone, Aldrich Chemicals) reagents. Analytical reagent grade TOPO and TTA were dissolved in ethyl alcohol since they were not soluble in water. TTA and TOPO were used to chelate europium ion and form a Eu-TOPO-TTA complex. The Eu-TOPO-TTA complex made the Eu^{+3} ion fluoresce. An emission filter, TRITC/Rhodamine (578-632nm) was modified by changing its original excitation filter with a UV DAPI/Hoechst/AMCA excitation filter (340-380nm). A UV light source was attached to the inverted microscope (Nikon TXE 300). After 15 min., the sample was flooded with pH 4.7 10mM acetate buffer and the sample was viewed under the microscope. The image viewed under the microscope was captured using a CCD camera (Princeton Scientific Instrument) mounted to the inverted microscope. The camera was

connected to a MacG3 computer with an IPLab Spectrum (Scananalytics Software) image analysis software. Camera exposure was set to 600 ms. A 4x objective lens was used to view the samples under the microscope.

Salt Soluble Protein Recovery

The strips of meat sections were individually rinsed with deionized water, and the rinse contained with the same amount of 0.6M of NaCl solution. The 0.6M of NaCl solution was added to the rinse liquid to ensure solubilization of salt soluble protein in the extract. Unmarinated breast meat fillet was sectioned similarly as the previous to serve as a control. Furthermore, 20 percent deionized water was added separately to two batches of breast meat fillet. One of the batches was tumble marinated (in-bag) at 10 RPM with 25 inches Hg to 4^{o}C and the other to 10^{o}C as a positive control (no salt and phosphate was added). The rinse was then filtered through a Whatman #4 filter and 1 ml. aliquot of the extract was assayed for protein by the Biuret method (Gornal et al. 1949). Protein concentration was determined from absorbance at 550 nm using a spectrophotometer (Spectronic Genesys 2) and a standard curve of Bovine Serum Albumin (Sigma Chemicals). Salt soluble protein content was reported in mg/mm^2 of marinated raw chicken breast sample.

RESULTS AND DISCUSSION

The trivalent Eu^3 ion was effective in tracing marinade diffusion in the meat. The stable fluorescence of chelated Eu^{+3} ion permitted viewing the depth of penetration under the microscope. The presence of TOPO as co-ligand effectively blocked the formation of a complex between Eu-TTA and phosphate. In the absence of TOPO no fluorescence can be observed. The phosphate-Eu-TTA complex quenched excitation energy from Eu (Schaff et al. 1969). According

to Arnaud and Georges (2003), Eu has nine ligands and TTA has only six or eight oxygen atoms available for coordination. Addition of TOPO provided a source of oxygen to form $Eu(TTA)_3(TOPO)_2$. The 0.005 M TOPO and 0.01 M TTA acidified to pH 4.7 were sufficient for complexation with Eu^{+3}. Increasing pH to 6.8 using the same buffer did not result in fluorescence. TTA acts as the chromopore and energy donor, while TOPO completes the ligand coordination to Eu^{+3} the fluorescing agent (Arnaud and Georges 2003).

Fluorescence of the Eu^{+3} marker in marinated chicken breast was brighter in the areas closer to the surface than in the meat interior. By positioning the UV light to shine through the sample and moving the light across the sample, Eu^{+3} was undetected in the interior areas. Thus, Eu in the marinade may not reach sections deep within the marinated meat. Fig. 4.1 and 4.2 shows the fluorescence captured by a CCD camera in tumble marinated meat sample at 4^oC. Absorption of marinade was detected to be limited to about 3.8 mm on skin side and 3.0 on skin side when marination was done at 4^oC. The depth of marinade penetration in vacuum tumbled meat at 10^oC was found to be deeper both for the skin side (Fig. 4.3) and bone side (Fig. 4.4) than the meat samples marinated at 4^oC. Results found in the present study were deeper than reported by Kay (2001). Only to 2 mm when salt and phosphate were in the marinade. Kay (2001) used high molecular weight dyes (flourescein and Lucifer yellow), which diffused slower than the Eu^{+3} used in the present study. Kay (2001) used a 7 percent salt marinade compared to 6 percent in the present study. Therefore the use of Eu^{+3} as a tracer for marinade penetration in vacuum tumbled marinated chicken breast is probably more indicative of marinade penetration in the meat than the use of dyes with high molecular weights. In addition, the limited detection of penetration seen on flourescein and Lucifer yellow dyes used by Kay (2001) may be attributed to higher interaction of the dyes to the proteins in muscle. On the other hand, Eu^{+3}

maybe more inert and interacts lesser to meat proteins, thus detection in the meat muscle was found deeper than the flourescein and Lucifer yellow dyes.

Meat temperature during vacuum tumbling significantly affected diffusion of marinade in the chicken breast meat. Marinade penetration (Figs. 4.1 to 4.4) was as high as 4.6 mm at 10^{o}C compared to 3.38 mm at 4^{o}C. Although marinade surrounded both sides of the meat, penetration was deeper from the bone side of the breast fillet compared to that on the skin side which may be attributed to the presence of perimysium and epimysium of the breast meat fillet. Both perimysium and epimysium stromal proteins of the chicken breast fillet could act as barrier and hinder marinade to penetrate into the interior part of the muscle.

The amount of salt soluble protein recovered near the surface on bone side of the fillet was low on chicken meat marinated at 10^{o}C but there were more salt soluble protein in the inner sections. More salt soluble protein was found in sections the same distance from the surface of meats marinated at 4^{o}C compared to 10^{o}C, up to 3mm depth from surface. The elevated temperature of the meat must have made the meat matrix more permeable which permitted salt to enter the meat matrix to solubilize the salt soluble protein. Meat is more pliable at higher temperature which means the meat muscle is more relaxed and less constricted. In addition, the combined vapor pressure of water in the meat and pressure of dissolved gases makes the meat expand opening up more spaces for marinade to occupy.

The salt soluble protein content of sections from the surface towards the center of the meat is shown in Figure 4.5. Salt soluble protein solubilization was more up to 7 mm deep from the surface measured from the bone side of the fillet marinated at 10^{o}C. This compares with the 4.7 mm depth detection of Eu tracer at 10^{o}C and 3.4 mm at 4^{o}C. Solubilization of protein indicated that salt must have penetrated to depths beyond that penetrated by the tracer.

Concentration of salt soluble protein appeared to increase with increasing depths. This is more evident in meat marinated at 10°C compared to 4°C. In meat marinated at 10°C, the highest measured salt soluble protein concentration was at the 7 mm depth. It is not known if the salt soluble protein concentration will continue to increase beyond the 7 mm depth or whether it would decrease as the limit for salt penetration is exceeded. The half thickness of the breast fillets was about 10 mm. The limited detection of Eu^{+3} in the interior part of the muscle maybe also limited due to the exposure time was not enough to detect the lesser concentration of Eu^{+3} as it penetrated the meat muscle. Furthermore, the atomic weight of the Eu^{+3} ion is 151 compared to 23 for sodium, thus the difference in the size of the molecule could make a difference in the diffusion rates into the meat.

A possible explanation for the lower salt soluble protein solubilized near the surface is leaching of the solubilized protein out of the meat matrix. The viscosity of salt soluble protein solution increases with temperature which would facilitate leaching at higher temperatures. Thus, the high viscosity salt soluble protein extract at 4°C does not leach out easily and there is little difference in salt soluble protein content with depth when marination was carried out at 4°C. Xiong and Blanchard (1994) reported a significant decrease in viscosity of salt soluble protein solutions (10mg/mL protein in 0.6M NaCl/50 mM sodium phosphate, pH 6.0) approximately from 123 to 90 cPa at 4 and 10°C, respectively. At higher temperature, the internal voids in the meat is also more because of the pliability of the meat and the increased expansion at higher temperature, therefore, the salt soluble protein solution could leave the interfibrillar spaces easier, and leave the meat matrix entirely near the surface. At 4°C, more solubilized salt soluble protein was present on the surface compared to 10°C giving further support of the theory of salt soluble protein solution migrating out of the meat. This observation

was corroborated by the results of Rejt et al. (1978) on the decrease of protein in biceps femoris after vacuum massaging. Velinov et al. (1990), reported the movement of extracted proteins out to the surface of a tumble marinated whole muscle. There must be some interaction between salt penetration and salt soluble protein solubilization, if salt levels are low at certain depths at 4^{o}C, then extraction of salt soluble protein out of the meat matrix can be hindered. Furthermore, the solubilized protein remains in the meat near the surface because of slower leaching at 4^{o}C due to the high viscosity of the protein extract. Xiong and Brekke (1989) reported increased viscosity of salt soluble protein extracts at the increase of protein concentration, thus leaching of salt soluble protein will be impeded. The negative and positive control showed no difference in protein concentration (Fig. 5). This showed that part of the protein determined in samples tumble marinated with salt and phosphate may include the sarcoplasmic protein that is 25-30% present in myofibrils (Scopes 1970). Our data on marinade absorption in these experiments showed significantly higher ($P<0.01$) values of 17.48±0.12% at 4^{o}C and 17.9±0.1% at 10^{o}C. Deeper penetration of marinade in meat must play a significant role in the higher percent marinade absorption at 10^{o}C compared to 4^{o}C. The role of protein solubilized in the meat also is factor in marinade absorption since soluble proteins interact with water more favorably than the intact muscle (Xiong and Brekke 1989). Marination process technologists should balance the problem posed by thickness of the meat and the restrictions on penetration offered by the larger depth to the permeability of the meat matrix which would allow the solubilized protein to leave the meat matrix.

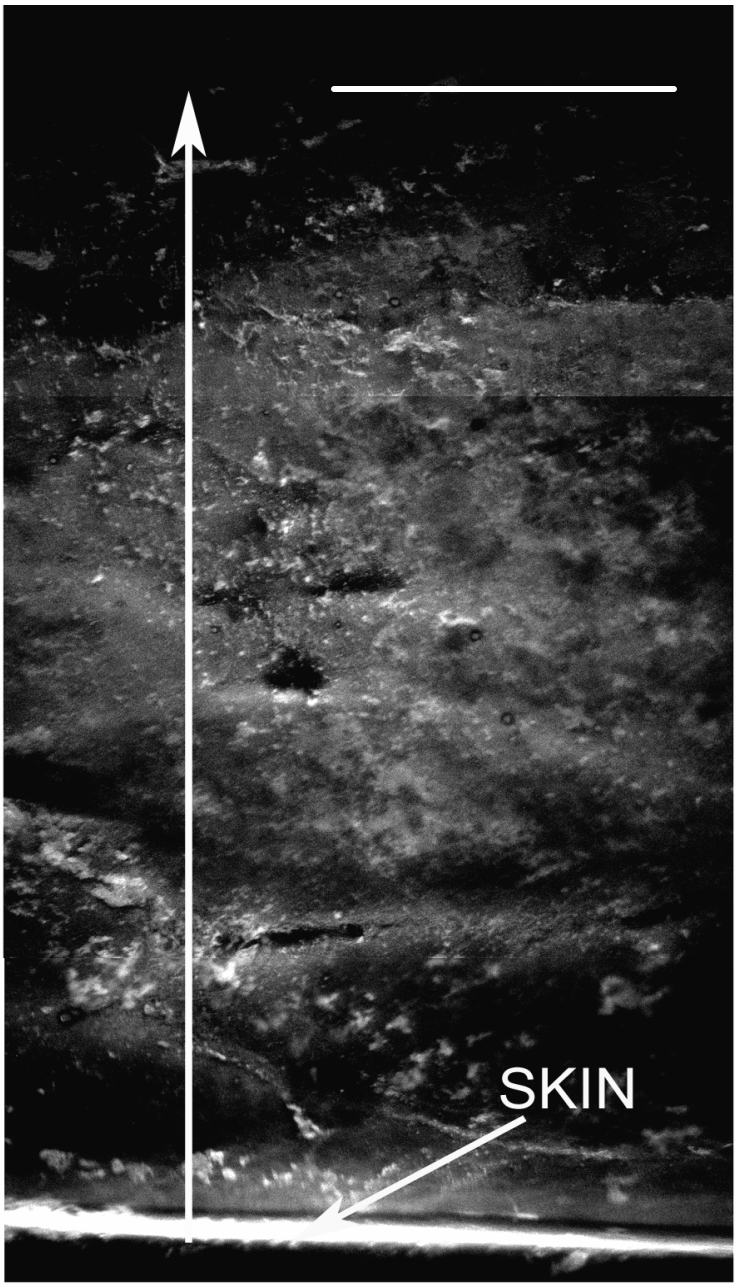
CONCLUSION

In conclusion, trivalent Eu^{+3} performed better in tracing marinade penetration in vacuum tumbled chicken meat compared to higher molecular weight tracing dyes. However, the

molecule size is still larger than the sodium ion, therefore depth of penetration as indicated by Eu^{+3} is still less than the depth where salt soluble protein solubilization has been observed. There is a significant difference in marinade absorption with meat temperature during marination.

Figures 4.1. Photomicrographs of raw chicken breast fillet marinated at 4^{o}C with 6.0% NaCl, 2.1% sodiumtripolyphosphate, and 0.001M europium. The line bar indicates the portion of the frame which exhibited fluorescence. The bottom frame represents the surface of the skin side of the fillet and the upper frames progressively show sections towards the interior of the fillet. Fluorescence was evident to a depth of 3.38mm. Bar = 1mm.

4.1)



Figures 4.2. Photomicrographs of raw chicken breasts marinated at 4^{o}C with 6.0% NaCl, 2.1% sodiumtripolyphosphate, and 0.001M europium. The line bar shows the progression of marinade diffusion in the meat. 2a is the surface on the bone side of the breast fillet. 2b and 2c show the continuation of the marinade penetration in the meat. Fluorescence was observed to a depth of 3.0mm. Bar = 1mm.

4.2)

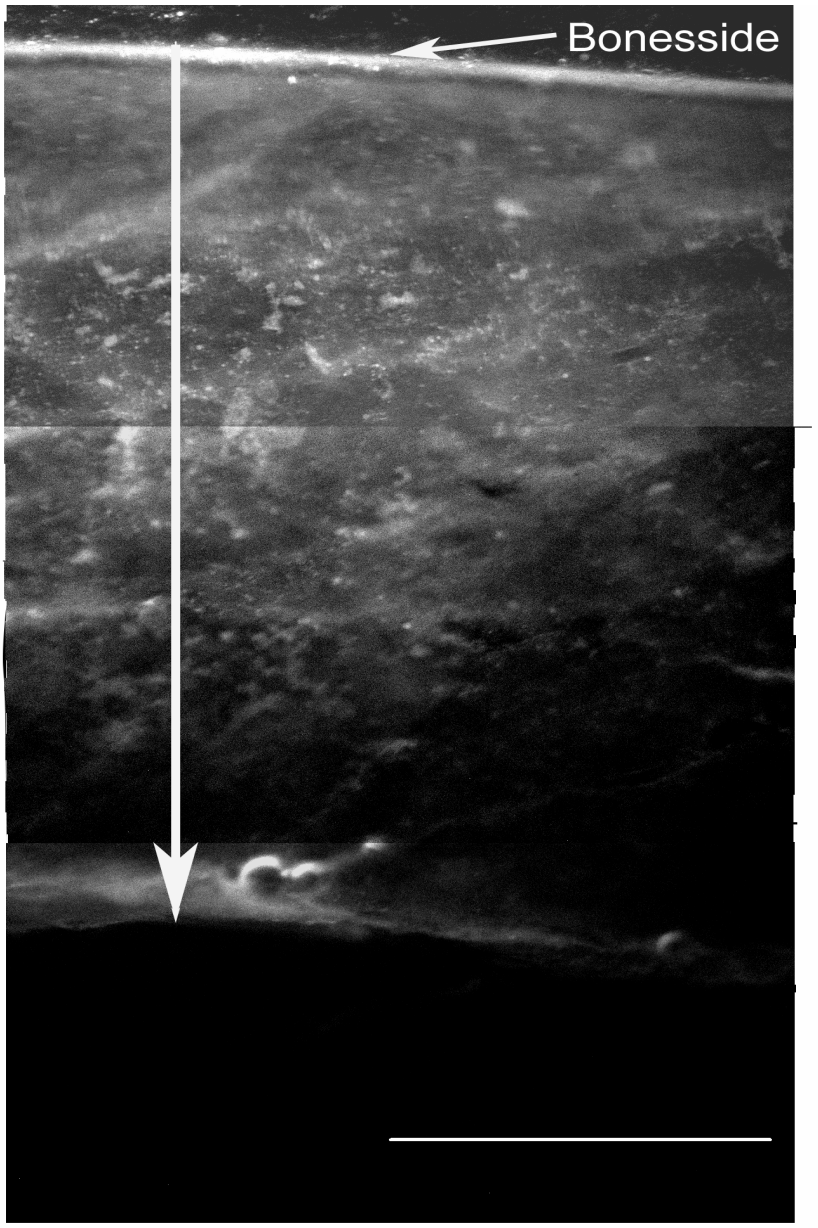
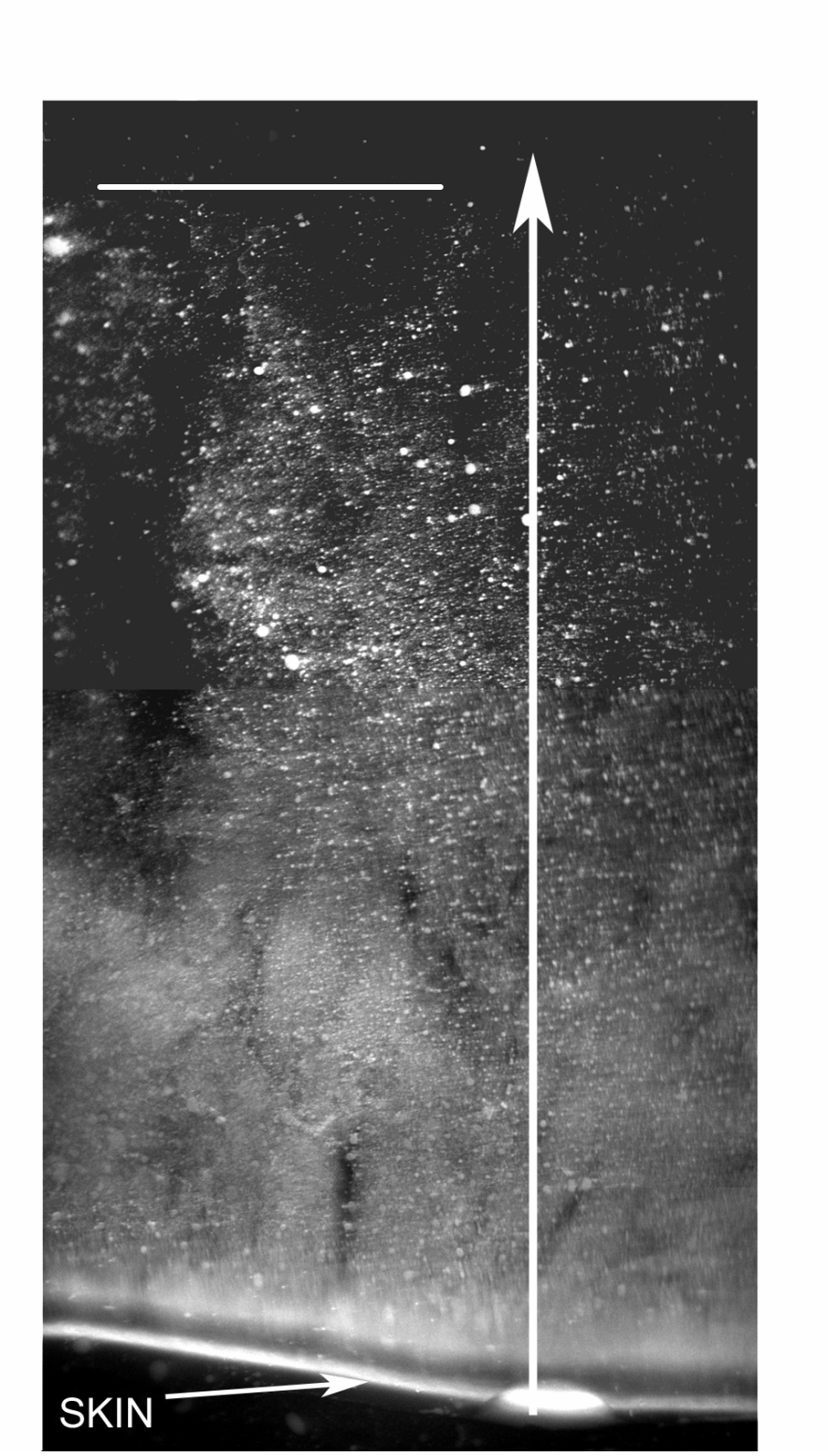


Figure 4.3. Photomicrographs of raw chicken breasts marinated at 10^{o}C with 6.0% NaCl, 2.1% sodiumtripolyphosphate, and 0.001M europium. The line bar shows the progression of marinade diffusion in the meat. 3c is the skin side of the breast fillet. 3a and 3b show the continuation of marinade penetration in the meat. Fluorescence was observed to a total depth of 3.61mm. Bar = 1mm

4.3)



Figures 4.4. Photomicrographs of raw chicken breasts marinated at 10^{o}C with 6.0% NaCl, 2.1% sodiumtripolyphosphate, and 0.001M europium. The line bar shows the progression of marinade diffusion in the meat. 4a is the surface on the bone side of the fillet. 4b, 4c and 4d show the continuation of the marinade penetration in the chicken breasts. Fluorescence was observed to a total depth of 4.62mm. Bar = 1mm

4.4)

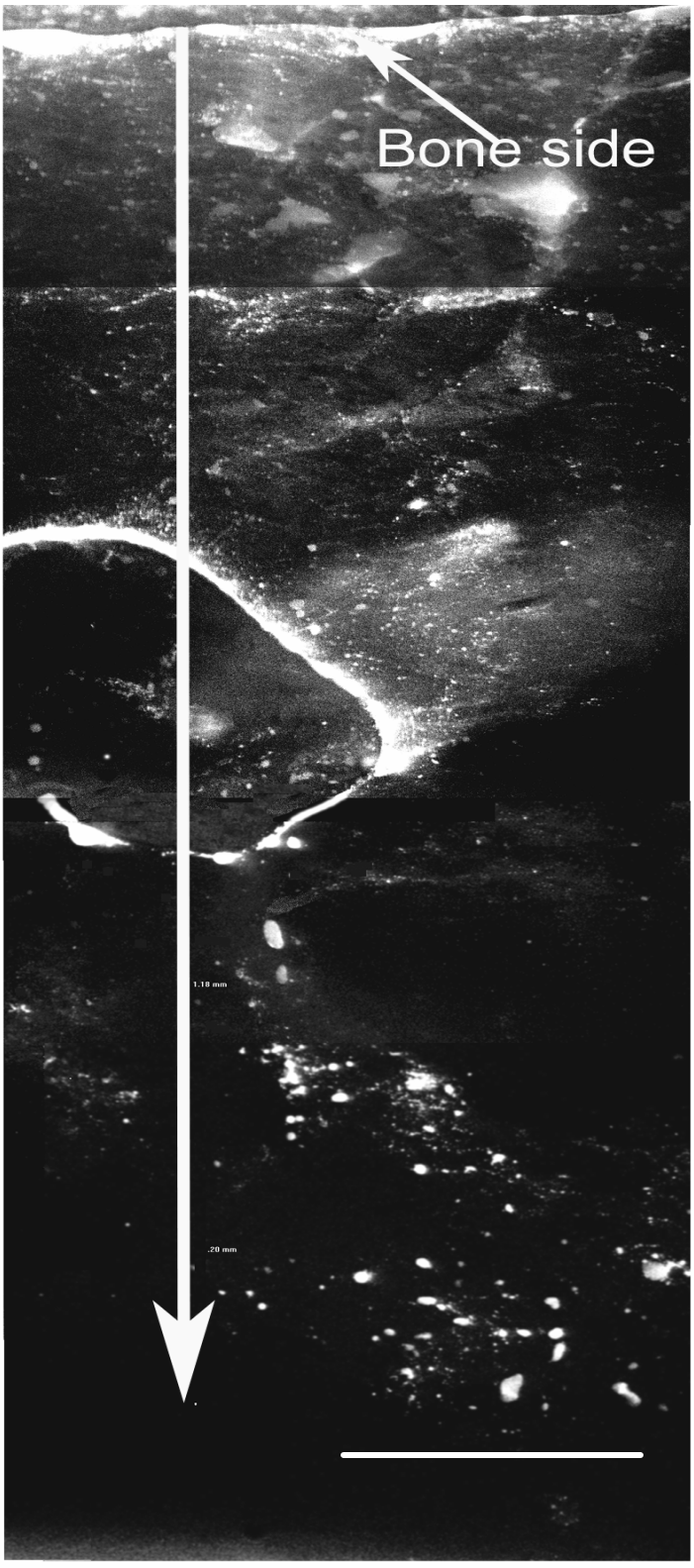
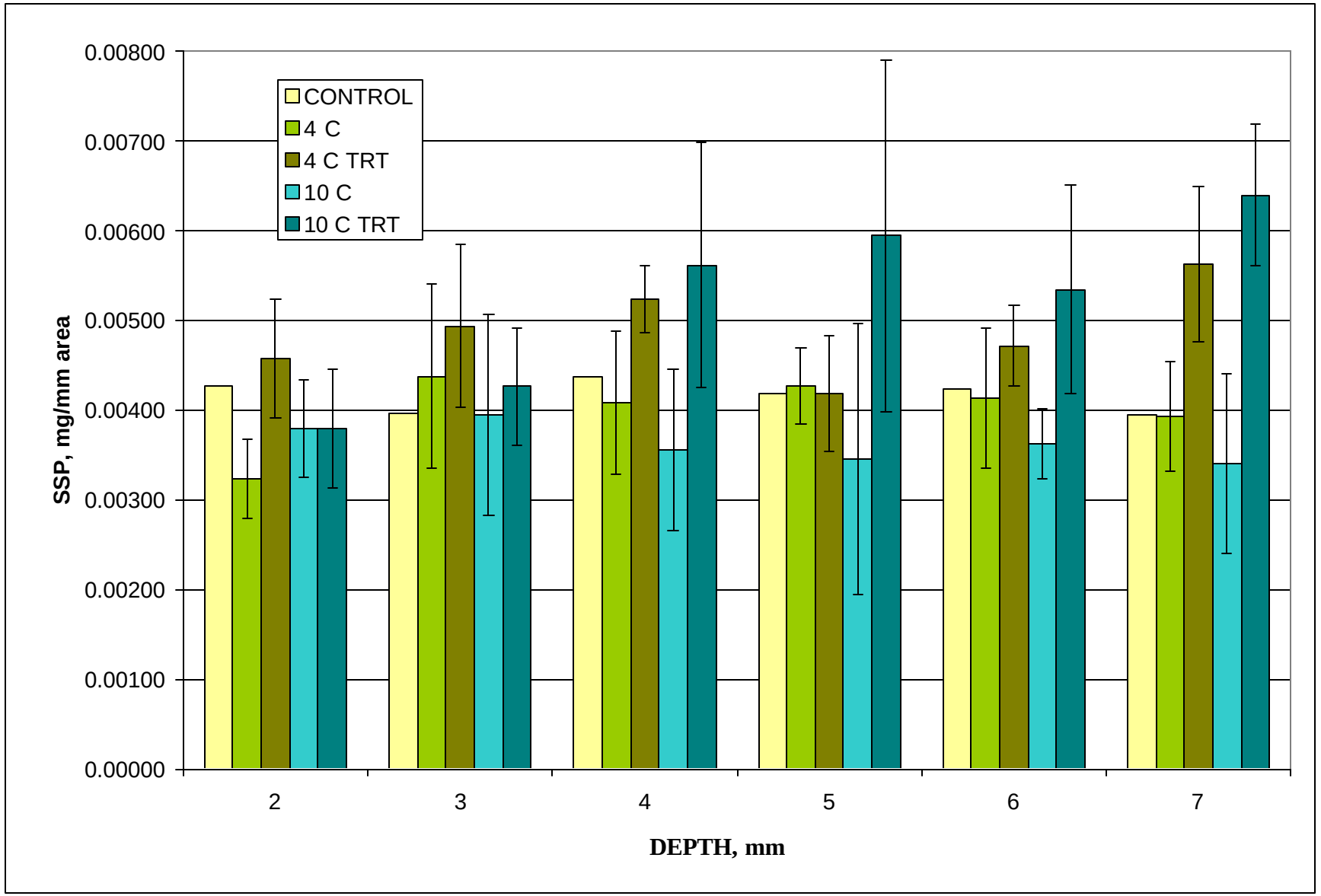


Figure 4.5. Recovered salt soluble protein from different depths in marinated raw chicken breasts was collected starting from the skin opposite side of the chicken breast sample. Control – raw unmarinated; 4C = marinated with deionized water only at 4^{o}C; 4C TRT – marinated with 6% NaCl and 2.1% sodiumtripolyphosphate at 4^{o}C; 10C- marinated with deionized water only at 10^{o}C; and 10C TRT - marinated with 6% NaCl and 2.1% sodiumtripolyphosphate at 10^{o}C at 10 RPM and 25 in. Hg vacuum.


0.00800
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0.00600
0.00500
0.00400
0.00300
0.00200
0.00100
0.00000
SSP, mg/mm area
CONTROL
4 C
4 C TRT
10 C
10 C TRT
2
3
4
5
6
7
DEPTH, mm

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CHAPTER 5

CONCLUSION

Complete denaturation of myoglobin pigments in chicken meat did not necessarily eliminate pink color. Pink color in cooked meat was influenced by the ORP value of the meat during and after cooking conditions rather than the incomplete denaturation of myoglobin pigment. Increasing salt concentration in marinade led to the increase of pink color intensity in cooked meat. Furthermore, increasing pH enhanced pink color intensity in meats. Appearance of pink color to otherwise perceive cooked meat, i.e. the cooked meat had brown-grey appearance, was made possible by exposure to reducing agents. Negative ORP values were observed to induce pink color appearance in cooked meat.

Temperature of marination process had significant impact in percent marinade absorption, percent cook yield, and percent expressible moisture. Percent cook yield and percent expressible moisture were found highest in chicken breasts marinated at 12^{o}C for 10 min and 4^{o}C for another 15 min. Furthermore, percent marinade absorption was highest when chicken breast meat was marinated at 12^{o}C for 25 min. In addition, Eu^{+3} was detected deeper in the interior part of the chicken breast fillet than fluorescein and Lucifer yellow dyes. Marinade penetration was deeper in meat marinated at higher temperature and had lesser amount of recovered salt soluble protein near the meat surface. Thus, Eu^{+3} may have penetrated the meat matrix deeper than what was detected. Detection of Eu^{+3} was only limited to about 4.6 mm depth of the chicken breast meat from its surface at 10^{o}C and 3.6mm at 4^{o}C.

In order to alter meat conditions and to influence the disappearance of pink color, flavor intensity, cook yield, and water holding capacity, marinade must be homogeneously distributed

in the meat. More work should be done in investigating the effectiveness of a two step marinade delivery system employing marination injection and vacuum tumbling marination to increase marinade functionality in marinated meats, as opposed to vacuum tumbling marination only.