FACTORS, PREVENTION, AND PREDICTABILITY OF THE PINK COLOR DEFECT IN UNCURED FULLY COOKED CHICKEN BREAST MEAT

by

CLINT THOMAS WILLS

(Under the Direction of A. Estes Reynolds)

Abstract

The occurrence of the pink color defect in poultry white meat results in serious economic losses to processors because of the association with undercooking by the consumer. A reduction in the occurrence of the pink color defect can be achieved by carefully controlling the processes to which live birds are exposed, using tailored marinade formulations to control the physical and chemical characteristics of the muscle, and monitoring the quality of water used in processing applications. A pinking prediction equation was established by utilizing the individual cooked muscle pH and oxidation reduction potential (ORP) to determine the probability of pink color formation. The ultimate method for controlling the pink color defect would be to sort raw chicken breast by color (pH) to further utilize marinade formulation to minimize the probability of pink color formation as well as account for yield factors.

INDEX WORDS: Chicken breast, Pink color defect, Predictability, pH, Oxidation Reduction Potential (ORP), Yield
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DEDICATION

This thesis and the work it encompasses is dedicated to my mother, Carolyn Wills, father, George Wills, brother, Jared Wills, and Grandmother, Nannell Dent. The love and support of my family has helped to deliver me to the point in my life I am currently at and with out their help none of my scholastic achievements would have been possible. A special thanks is also extended to my close friends, who have also provided support. Thanks to everyone who made this achievement possible.
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CHAPTER 1
INTRODUCTION
1.1 Statement of the Problem

In the United States over the past thirty years, the poultry industry has experienced dramatic changes. The type of products consumers are in the market for at the present time has completely changed from years ago. The market for poultry products has increased by 510 percent in slaughtered broilers or young chickens from 1960 to 1998 (Ollinger et al., 2000). The whole, ready-to-cook carcass was the preferred product being purchased by consumers and being marketed by the processors in the past. In 1962, whole, ready-to-cook carcasses accounted for 87 percent of the processed broiler market. However, today consumers are in the market for convenient, healthy products, which in turn has dropped the whole, ready-to-cook carcass market to only 13 percent in 1997 (Ollinger et al., 2000). In the demand for a quick, easy, and healthy meal, the poultry industry has had to adapt to the growing market by shifting most of the production to cut-up, debone, and value added further processed products. The shift in the market is one of the major forces increasing broiler production rates by approximately five percent each year (Ollinger et al., 2000).

The majority of households today are dual income families, with both spouses fully engulfed in their careers. In the past, most families had a parent that was able to stay home to care for children and daily chores, such as cooking. In today’s society, both parents come home from a full day at the office or job site to a second job of caring for the household. The days of having hours to prepare meals are coming to an end and consumers are now looking for convenient meal solutions for their families. The market has shifted and so has the poultry industry. However, there has been a defect occurring sporadically in the further processed products being produced by the meat industry, especially the poultry industry, which has contributed to major economic losses.
This defect is called the Pink Color defect. This defect is the development of a pink color, tinge, spot, or ring on the interior portions of uncured, fully cooked poultry meat products. The pink color that appears gives the appearance of an undercooked and unsafe product. This color defect has had a direct economic impact on poultry processor because of consumer rejection and discounting by commercial buyers (Schwarz et al., 1997). The color of a product is the easiest way for consumers to judge the quality of that particular product. The presence of the pink color defect will cause consumers to reject the product associating it with poor quality, which in turn reflects badly on the processor. Another detrimental problem caused by the pink color defect is excessive cooking by consumers thinking the product is not fully cooked. This will cause a poor quality product because the tenderness and juiciness is reduced by the excessive cooking and once again will reflect badly on the processor. Many years of research have been conducted on this subject to understand the causes of the pink color development and ways to reduce or inhibit this defect.

1.2 Objectives

1. Discuss possible factors contributing to the formation of the pink color defect

   • Preslaughter factors
   • Stunning techniques
   • Various classes and types of pigments
   • Non meat ingredients and cooking methods
   • Nitrate/Nitrite contamination
   • Irradiation

2. Use of marinade formulations to control muscle ionic strength levels to reduce pink color defect in uncured fully cooked chicken breast meat.
• Measure chemical and physical changes of the muscle through the raw, marinated, and cooked phases of the muscle (pH, ORP, Color, Yield)

• Develop an equation to predict the chance of pink color formation as a function of cooked muscle pH and ORP.

3. Examine the quality of water being used within poultry plants across the State of Georgia

• Possible sources of nitrate/nitrite contamination

• Measure nitrate/nitrite concentration and hardness levels of water used in poultry plants

1.3 References


CHAPTER 2

LITERATURE REVIEW:

FACTORS ASSOCIATED WITH PINK COLOR FORMATION
2.1 Introduction

The pink color defect is the development of a pink color, tinge, spot, or ring on the interior portions of uncured, fully cooked poultry meat products. The pink color that appears gives the appearance of an undercooked and unsafe product. This color defect has had a direct economic impact on poultry processor because of consumer rejection and discounting by commercial buyers (Schwarz et al., 1997). The color of a product is the easiest way for consumers to judge the quality of that particular product. The presence of the pink color defect will cause consumers to reject the product, associating it with poor quality, which in turn reflects badly on the processor.

The cause of the pink color defect in uncured fully cooked poultry products has been attributed to various factors. The research done over the years has attributed the pink color defect to be caused by (1) preslaughter factors such as heat and cold stress, gaseous environment, genetics, feed, hauling, and handling (Froning and Hartung, 1967; Froning et al., 1968a, 1969a, 1978; Babji et al., 1982; Ngoka et al., 1982; Sackett et al., 1986), (2) stunning techniques (Ngoka and Froning 1982; Froning, 1995; Young et al., 1996a; Craig et al., 1999), (3) various classes and types of pigments (Fox, 1966; Tappel, 1957; Ledward, 1974; Livingston and Brown, 1981; Izumi et al., 1982; Cornforth et al., 1986; Girard et al., 1989; Ghorpade and Cornforth, 1993; Smith, 1998), (4) current industry procedures including the use of nonmeat ingredients and cooking methods (Pool, 1956; Froning et al., 1968c; Helmke and Froning, 1971; Janky and Froning, 1973, Trout, 1989; Ahn and Maurer, 1989a, 1990a,b; Claus et al., 1994; Cornforth et al., 1998), (5) incidental nitrate/nitrite contamination through water supply, diet, freezing and processing equipment, and processing ingredients (Froning et al., 1968b, 1969b; Mugler et al., 1970; Nash et al., 1985; Ahn and Maurer, 1987; Fleming et al., 1991; Heaton et al., 2000), and
(6) irradiation of precooked products (Nam and Ahn, 2002a,b). The in situ conditions of the meat, which are the original native conditions of the meat as they are found in the animal, are altered by various factors, identified previously, to cause changes within the muscle, such as pH, degree of denaturation, reactivity of endogenous meat compounds, and reducing conditions. The reactivity, chemical state, and structure of the native muscle pigments are also affected by these various factors, which could cause the occurrence of the pink color defect (Holownia et al., 2003). The color of meat is attributed to the light-scattering matrix of cellular material, myofibrillar proteins, connective tissue, and light-absorbing pigments (MacDougall, 1970; Swatland, 1983). The muscle fibers in dark colored meat are packed tighter together because they are associated with more bound water in the muscle than light color meat. This increases translucence and reduces the lighter scattering capability of the meat allowing a deeper penetration of the light to be absorbed by the available myoglobin, which produces a dark color. The light scattering and absorbing properties of meat are affected by changes in the in situ conditions of the product (Holownia et al., 2003). Therefore, the final color of raw meat products is determined by the changes of in situ conditions that are caused by many intrinsic and extrinsic factors.

2.2 Preslaughter Factors

Preslaughter conditions that animals are exposed to during growth and shipment to processing plants can have adverse effects on the quality of meat produced from that animal. Harsh conditions that induce stress upon the animal during growth, transportation, and holding, such as periods of high, low, or wide temperature fluctuations, gaseous environment, feed and water deprivation, hauling, and handling, will cause a low quality meat to be produced. The pale, soft, and exudative (PSE) condition caused by low meat pH and the pink color defect
caused by high meat pH are the best examples of stress effects on muscle quality (Wood and Richards, 1975).

In studies done by Wismer-Pedersen (1959) and Forest et al. (1963), periods of wide temperature fluctuations or high temperatures increased the occurrence of the PSE condition in pork. Pigs held at elevated temperatures immediately before slaughter experienced a rapid rate of postmortem muscle glycolysis as indicated by a rapid decline in muscle pH and decreased muscle color intensity (Sayre et al., 1963). Dark cutting meat and high pH were exhibited in cattle exposed to low winter temperatures prior to slaughter (Lawrie, 1966). In chicken broiler muscle, Wood and Richards (1975) observed cold stress lengthened the period of postmortem glycolysis while heat stress hastened the rate of postmortem glycolysis. Similar results were found by Froning et al. (1978) and Lee et al. (1976), in which preslaughter heat stressed birds produced a lower pH than birds observed under cold stress conditions. Also, in experiments done by Babji et al. (1982), breast meat from heat stressed birds had significantly lower raw and cooked pH values, lower water holding capacity, higher cooking loss, and was less desirable than the breast meat from the control or cold stressed birds. In experiments conducted by Froning et al. (1978) significantly lower L* (darker) and higher a* (redder) values were found in white meat from heat stressed birds. Prolonged exercise raised the pH value and juiciness of meat was reported by Briskey et al. (1959). The pH of the meat is greatly affected by exposure to stressful environments such as high and low temperatures.

Poultry meat color is markedly affected by the final pH of meat after slaughter. Meat with low pH values exhibit a pale, less red color where as higher pH meat gives a darker, redder color (Babji et al., 1982). Muscle pH and meat color are highly correlated as shown by previous research. Breasts with low muscle pH are commonly observed to have a light color, whereas
breast muscles with a high pH are shown to be dark in color (Fletcher, 1999). The final pH of the breast muscle is directly related to the biochemical state of the breast at the time of slaughter and during rigor mortis development. The light reflectance property of the breast is influenced by the pH. The biochemical state of the breast muscle is determined by the rate of postmortem glycolysis. An increase in the rate of postmortem glycolysis will cause a rapid pH decline and will cause the breast to have a light pale color (Babji et al., 1982). The pH decline is a product of the lactic acid produced during postmortem glycolysis. As birds are held at higher temperatures prior to slaughter, the rate of postmortem glycolysis will increase to produce meat with pale color and low pH (Wood and Richards, 1975). Cold stress prior to slaughter will reduce the rate of postmortem glycolysis and produce a meat with a darker color and a higher pH. The changes in pH are all due the conditions the bird experienced prior to being slaughtered. The importance of proper procedures preslaughter is essential in producing a high quality product that has normal color and functionality.

The type of gases birds are exposed to during growth and preslaughter procedures could be another cause of the pink color formation occurring in fully cooked poultry products. Hydrogen sulfide, methane, ammonia, carbon monoxide, and carbon dioxide are the predominant gases found in poultry houses during growth of birds (Day et al., 1965; Taeginides and White, 1969; Ludington et al., 1971). Ammonia is produced in houses mainly from the excretion of the birds. The presence of high concentrations of ammonia within poultry houses significantly increased the amount of carcass condemnations due to undergrades and breast blisters (Quarles and Kling, 1974). In research done by Sackett et al. (1986), the presence high concentration of ammonia (75 ppm and 100 ppm) caused birds to become more restless and excited than birds not
exposed to ammonia. However, the exposure to ammonia did not have an effect on the color or pigment concentration in muscle.

Carbon dioxide is present in normal atmospheric air at 300 ppm and is an odorless gas (Handbook of Chemistry and Physics, 1978). Carbon dioxide is predominantly produced by respiration of the birds in enclosed houses. In poultry houses, carbon dioxide concentrations range from 300 to 2,500 ppm during the winter and 400 to 1,300 ppm during the summer months (Nakaue et al., 1981). High concentrations of carbon dioxide can be fatal due to asphyxiation and deprivation of oxygen. In humans, carbon dioxide concentrations of 40,000 ppm can cause increased depth and rate of respiration, and concentration above 100,000 ppm may cause dizziness and even unconsciousness (Livestock Waste Facilities Handbook, 1975). In research conducted by Sackett et al. (1986), carbon dioxide levels between 2,500 and 10,000 ppm increased the pH of raw breast muscle. The increased carbon dioxide exposure caused the utilization of stored glycogen in the bird in order to adapt to the stress, which in turn after death resulted in less glycogen for postmortem glycolysis and less lactic acid being produced, thus yielding a higher pH and darker colored meat.

Incomplete combustion of fuel during improper operation of heaters is responsible for the production of carbon monoxide in poultry houses (Sackett et al., 1986). Birds can also be exposed to carbon monoxide from the exhaust of trucks used in transportation from farm to the plant (Froning et al., 1969a). The a* (redness) values increased in raw poultry meat from birds exposed to carbon monoxide prior to slaughter. As the concentration of carbon monoxide is increased, the redness of the meat increased. Carbon monoxide will react and complex with myoglobin and hemoglobin to form carboxymyoglobin and carboxyhemoglobin, respectively. A bright cherry red color is a characteristic of these pigments and will likely impart a red color into
the meat tissues. Once carboxymyoglobin and carboxyhemoglobin pigments are formed in poultry meat, the pigments will likely remain stable throughout the cooking procedure, and influence the detrimental pink color defect occurring in uncured fully cooked poultry products (Froning et al., 1969a). However, in a study by Sackett et al. (1986), the carboxymyoglobin and carboxyhemoglobin pigments were formed upon exposure to carbon monoxide, but did not remain in the cooked product. The color of fully cooked poultry products were affected by carbon monoxide exposure causing a pink color to be formed in the product in research completed by Froning et al. (1969a). In birds exposed to carbon monoxide at the level of 1,000 ppm, the pH was increased in raw muscle when compared to lower levels of carbon monoxide exposure. The birds exposed to levels of carbon monoxide up to 750 ppm appeared to be excited and stressed which caused a lower pH in the muscle. The higher level of 1,000 ppm caused birds to become somewhat anesthetized and more tranquil prior to slaughter and is the reason for a higher pH than the stressed excited birds (Sackett et al., 1986). The exposure of birds to carbon monoxide can be a cause of the sporadic occurrence of the pink color defect. The environmental condition present inside poultry houses and on trucks during transportation should be monitored and controlled. The proper ventilation of poultry houses is needed to produce a quality bird, which will in turn produce a quality product.

The quality of meat produced from animals can be influenced by their feeding or feed withdrawal times. In research completed by Ngoka et al. (1982), a significant decline in final pH was found in poultry that was not removed from feed prior to slaughter as compared to birds properly removed from feed. The same results were discovered for pigs. A decline in the final pH was discovered for pigs not removed from feed prior to slaughter as compared to pigs removed from feed. The rapid decline in final pH of animal not removed from feed prior to
slaughter is a result of the rapid onset of rigor due to depletion of glycogen. The meat produced from animals not properly removed from feed experienced low muscle pH, low moisture content, and poor water holding capacity. Therefore, a poor quality meat can be produced if the animal is not properly removed from feed prior to slaughter.

2.2 Stunning Techniques

Stunning is used to render the animal unconscious prior to slaughter to reduce the amount of struggle and stress. A struggling animal will produce a low quality meat due to bruises on the meat as well as a low final pH due to an increase in the rate of postmortem glycolysis. The stunning techniques used are mainly used to reduce the amount of stress on the animal prior to slaughter, so a higher quality meat will be produced. In research done by Thomson et al. (1986), the pH values of muscles from stunned birds was considerably higher than muscles from unstunned chickens immediately postmortem. It has been suggested that ante-mortem aerobic oxidation of glycogen is accelerated by the stunning process (Lee et al., 1976). This in turn causes less glycogen to be available for postmortem anaerobic glycolysis and consequently diminishes the production of lactate and pH decline. In research done by Young et al. (1996b), a higher ultimate meat pH was reached when increasing ante-mortem stunning time because the postmortem muscle glycogen levels were reduced by the increase in stunning time. The results showed that for each second increase in stunning time the pH is increased by 0.016 units. In this same experiment, it was determined that color of the meat was not affected by stunning time. However, the shear value of the meat was increased when birds were stunned for more that four seconds, which means a low quality tough meat was produced. Birds stunned from six to ten seconds were evaluated by a consumer panel as slightly tough in an experiment done by Lyon and Lyon (1991). In experiments done by Craig et al. (1999), birds were exposed to a high
current stun, low voltage stun, or unstunned prior to slaughter. The birds that experienced the high current stun had a significantly higher raw muscle pH (6.41) than the low voltage stun raw muscle pH (6.17) or the unstunned group raw muscle pH (6.10). The high current stun and low voltage stun produced a higher redness (a*) value than the unstunned birds. The application of stunning could be a cause of the appearance of the pink color defect being currently found in fully cooked poultry meat. In contrast to the previous research, Ngoka et al. (1982) suggested that birds allowed to freely struggle during slaughter may produce an abnormal red coloration in the muscle due to higher myoglobin concentrations within the muscle. These findings can be traced back to the application of stress and excitation to the animal to produce a darker, redder muscle. This could also be a reason for the formation of pink color within fully cooked poultry meat.

2.4 Various Classes and Types of Pigments

Myoglobin is the principal heme pigment influencing meat color; along with cytochrome c and hemoglobin, which also contribute to the overall color of meat products (Ngoka and Froning, 1982). These heme pigments and their reactions with other constituents in the muscle have received blame for the occurrence of the pink color defect in fully cooked poultry meat by several researchers. The main pigment of concern to meat scientist and processors is myoglobin. Myoglobin is a monomeric, globular protein consisting of a heme group surrounded by a globin moiety and has a molecular weight of approximately 18,000 (Livingston and Brown, 1981; Seideman et al., 1984). The heme portion of myoglobin contains a centrally located iron (Fe) atom within four pyrollic rings (Seideman et al., 1984). The valence state of the iron (Fe) atom and the ligand binding at the free bind site of the heme is of prime importance in determining the color of meat (Livingston and Brown, 1981; Seideman et al., 1984). The heme group of the
The myoglobin molecule is surrounded and protected by the globin moiety, which is a long chain protein (Clydesdale and Francis, 1971). The concentration, oxidative state, and reactivity of myoglobin are factors that may affect meat color.

The concentration at which myoglobin exist in the muscle determines the color of the meat. Beef is much darker in color as compared to poultry meat because the concentration of myoglobin is much greater. The concentration of myoglobin also varies within muscle type. The thigh muscle of a turkey contains about 10 times as much myoglobin as the breast muscle (Janky and Froning, 1973), which provides the reasoning for the difference between white (breast muscle) and dark (thigh muscle) meat. The meat with the lowest concentrations of myoglobin is pork and is the lightest in color. Muscles that are used frequently, such as thigh muscles and leg muscles in poultry for locomotion, will usually have a higher myoglobin concentration than muscles used less frequently, such as breast and wing muscles, because of the increased amount of oxygen required for the production of energy, therefore producing a darker colored meat (Seideman et al., 1984). In studies conducted by Seideman et al. (1984), more active animals such as those on pastures or free range birds produced darker colored meat with higher myoglobin concentration when compared to less active animals, such as those in a feedlot.

Ante-mortem factors such as stress, species, sex and age of animals, postmortem pH rate of decline and the final ultimate meat pH will also influence the color intensity of the meat (Seideman et al., 1984). The affects of stress on meat color is tremendous and was discussed above, animals that are exposed to stress and harsh factors immediately prior to slaughter will produce a meat with PSE conditions, which is pale in color, soft, and exudative. Animals that were stress for a prolonged period time prior to slaughter will produce a meat with DFD conditions, which is dark in color, firm, and dry. The sex of the animal is relative to color
intensity, which is also caused by differing concentrations of myoglobin. Meat derived from male animals is usually darker than meat from female animals due to a greater concentration of myoglobin (Seideman et al., 1984).

Immediate ante-mortem or postmortem conditions largely influence the pH of a muscle. A muscle with a low ultimate pH is produced from animals exposed to short term, violent stress immediately before slaughter or the carcass of the animal was slowly cooled after slaughter. A low ultimate pH is a characteristic of meat with PSE conditions, which is a result of rapid postmortem pH decline and high carcass temperatures. The low pH produced in this type of meat will cause a denaturation of sarcoplasmic and myofibrillar proteins, which will disrupt the muscle structure and cause the muscle fibers to become more “open” and scatter light to produce a meat paler in color. Also, the low ultimate pH will cause metmyoglobin to be formed by oxidation of the myoglobin fraction more readily than that of normal pH meat (Seideman et al., 1984). When a high ultimate pH is present in the muscle the opposite condition of PSE is usually formed, which is DFD (dark, firm, dry). This condition is a result of long term exposure to stress and depletion of nutrients, primarily glycogen. This condition in meat will produce a high ultimate pH along with a dark appearance. The DFD condition will occur in meat when there is not enough glucose or glycogen present in the muscle to lower the pH by glycolysis to lactic acid, which will in turn cause a high pH in the meat and the muscle fibers are swollen and tightly packed together forming a barrier to the absorption of light and diffusion of oxygen preventing the formation of the normal red color of meat known as oxymyoglobin (Seideman et al., 1984). Once again, the color of meat is related to the pre-slaughter conditions and stresses the bird experience prior to slaughter.
The color of myoglobin is dependant upon the oxidative state and is available in three color forms, which are deoxymyoglobin, oxymyoglobin, and metmyoglobin. The amount of oxygen present at the time the color of myoglobin is observed will determine which of the three forms will persist. In the absence of oxygen, myoglobin is in the reduced form called deoxymyoglobin and is purple in color. When in the presence of oxygen, “oxygenation” occurs, which allows the oxygen molecule to form a covalent bond with the free binding site of deoxymyoglobin (Seideman et al., 1984). This reaction will occur very rapidly because of myoglobin’s high affinity for oxygen (Pirko and Ayres, 1957). Oxymyoglobin is the desired form of myoglobin in red meat by the processor because of the red color, which is preferred by consumers to be the normal color of meat. The last of the three color forms that myoglobin can exist in is metmyoglobin, which is brown in color. Metmyoglobin is formed when low oxygen tension prevails and oxidation of the iron of the heme moiety occurs. The reactions that convert myoglobin to any of the three forms are reversible depending upon the conditions, low oxygen tensions will favor brown, metmyoglobin formation where as high oxygen tensions will favor the red, oxymyoglobin form. The deoxymyoglobin form will be predominant when oxygen is absent from the atmosphere surrounding the meat (Seideman et al., 1984).

However, any condition that causes denaturation of the globin moiety of oxymyoglobin, such as low pH, high temperature, ultraviolet light, and low oxygen tensions, will result in deoxygenation because the biological function of protecting the heme from undesirable reactions is lost (Walters, 1975). Once the heme protecting function is lost, oxidation of the iron in the heme moiety occurs resulting in deoxymyoglobin or reduced myoglobin. In the heme moiety, the iron molecule will lose an electron and the stable, original ferrous state (Fe ++ ) will be converted to the unstable, ferric state (Fe ++ +) (Seideman et al., 1984). Once in the ferric state,
myoglobin will spontaneously oxidize to metmyoglobin because the molecule is very unstable in this state. The oxygen utilizing enzymes inherent in the muscle tissues are the actual cause of the oxidation to metmyoglobin (Walters, 1975).

The activity of oxygen utilizing enzymes is increased with increasing temperatures, such as in cooking, and metmyoglobin is readily formed. Also, high storage temperature will increase the rate of oxidation of oxymyoglobin to metmyoglobin and reduce the case life or shelf life of a meat product based on color acceptability. The deoxygenation of red oxymyoglobin of case ready meat is reduced with low storage temperature because the activity of the oxygen utilizing enzymes is suppressed (Lawrie, 1974). The oxidation of myoglobin is also promoted by the presence of an acidic environment within the muscle (less than pH = 5.4). The globin moiety is denatured by the low pH, which will cause subsequent dissociation of oxygen from the heme to oxidize myoglobin to metmyoglobin (Siedeman et al., 1984). As the pH of a meat product decreases, the rate of oxidation is increased (Brooks, 1937). This is one of the reasons for low color intensity in meat with the PSE condition (Walters, 1975). The oxidation of myoglobin is detrimental to the color of a meat product, so careful attention of processors and retailers is need to combat any conditions that might cause denaturation of the globin moiety.

The key to understanding the color changes and reactions of myoglobin is the structure and chemistry of the iron atom located in the center of the heme moiety. The iron atom has eight valence electrons and is a third row transition metal, which means it contains unfilled energy levels below its valence electron levels. The absorption of visible light is attributed to the transition of electrons from filled orbitals to empty “3d” orbitals, which provides the color of myoglobin. Myoglobin in the in situ or native state contains an iron atom that has lost two electrons due to its low electronegativity. The iron is in the ferrous form (Fe ++), but due to
denaturation a third electron may be lost. This will change the iron to the ferric form (Fe ++ +), which is unstable and will bind with other ligands to increase stability (Livingston and Brown, 1981).

The structure of myoglobin consists of eight $\alpha$-helical segments. The $\alpha$-helical segments are folded to give a flattened, globular shape, which provides a cleft to protect the heme moiety (Livingston and Brown, 1981). The heme moiety is held in position within the cleft by 25 amino acid residue, which makes the heme difficult penetrate. The crack in the cleft that allows molecules, such as oxygen, to slide into the heme ring is small, thus protecting the heme from solvents and preventing the heme from binding with larger native muscle ligands. The cleft provides protection for the heme until the myoglobin molecule is denatured, which will result in an unfolding of the protein and exposure of the heme moiety (Price and Schweigert, 1987).

The myoglobin molecule usually has six ligands bound to the iron cation. The heme pyrrole nitrogens occupy four of the six coordination positions. The fifth coordination position (also called the proximal or axial position) in native myoglobin is occupied by histidine. The sixth coordination position is usually bound to oxygen, but the oxygen can be removed and replaced with other ligands small enough to enter the protective cleft unless the protein has been denatured. The only ligands that can enter the undenatured myoglobin cleft and bind to the sixth coordination position are oxygen ($O_2$), carbon monoxide (CO$_2$), and nitric oxide (NO). However, when myoglobin is in the deoxymyoglobin or reduced myoglobin form, the sixth coordination position is left open (Livingston and Brown, 1981).

Oxymyoglobin is formed when the sixth coordination position of undenatured myoglobin is bound to oxygen. The oxygen is bound in stable manner and the red oxymyoglobin form will persist until oxygen is removed from the atmosphere surrounding the molecule or oxidation.
occurs to form metmyoglobin, the brown derivative. Oxymyoglobin, deoxymyoglobin, and metmyoglobin have been previously discussed and are the usual forms of myoglobin unless the protein has been denatured or contamination with CO or NO has occurred (Livingston and Brown, 1981).

Carbon monoxide can bind tightly to myoglobin to form ferrous carboxymyoglobin, which provides a stable red color similar to that of oxymyoglobin. However, unlike oxymyoglobin, carboxymyoglobin is very stable and will remain intact once denaturation has occurred. Carboxymyoglobin can be a cause of the sporadic occurrence of the pink color defect because the red color of the meat will remain after denaturation of the protein, such as in cooking. The red color that remains will provide an undercooked appearance to the product even though the product has been fully cooked. The red color remains even after denaturation because carbon monoxide dissociates slowly from the ferrous myoglobin. Carbon monoxide dissociates about 1,000 times slower than oxygen and is stable, which means that the dissociated carbon monoxide will likely rebind to myoglobin thus producing the red color (Livingston and Brown, 1981). As described above, birds are being exposed to carbon monoxide during transportation and in poultry house, but the use of gas ovens may produce carbon monoxide that will bind with myoglobin prior to cooking forming carboxymyoglobin and the red color it imparts (Pool, 1956). The elimination of exposure to carbon monoxide is impossible, but consideration should be taken by processor to reduce the amount of exposure.

Nitric oxide myoglobin is a form of myoglobin that has received blame for being the cause of the pink color defect. The reaction of deoxymyoglobin with nitrite under anaerobic conditions will generate nitric oxide myoglobin. Nitric oxide myoglobin is similar to oxymyoglobin in color with the only difference being the nitric oxide bound to the sixth
coordination position instead of oxygen. Nitric oxide myoglobin is considered to be unstable in situ because oxygen will replace nitric oxide once it has dissociated from the myoglobin, but nitric oxide dissociates much more slowly than does oxygen, about one million times slower. The reason nitric oxide myoglobin can be a cause of the pink color defect is because once the protein is denatured; nitric oxide myoglobin becomes stable producing a bright red color (Livingston and Brown, 1981). The presence of nitrate and nitrite in processing water is the most likely place for contamination of the meat. Nitrite is the most important because it can readily be broken down to nitric oxide, which can react with myoglobin; however, nitrate can be broken down by microorganisms to nitrite and nitric oxide. The contamination of meat with nitrate and nitrite will be further discussed later in this paper and research was done to examine processing water from several processing plants for the presence of nitrates and nitrites.

Once the protein has been unfolded due to denaturation, the cleft that protected the heme is diminished exposing the heme moiety. The exposure of the heme moiety allows ligands to complex the iron center to form ferrohemochromes or ferrihemochromes, depending upon the state of the iron. Ferrohemochromes are complexes formed by ferrous (Fe ++ ) myoglobin with a non-oxygen sixth ligand. Ferrihemochromes are complexes formed by ferric (Fe +++ ) myoglobin with a non-oxygen sixth ligand. Several types of amino acid side chains can bind to the sixth coordination position of the heme moiety once the myoglobin molecule has been denatured. Different hemochromes are constantly interconverting because the exposed heme moiety can rapidly exchange ligands. The ferrohemochrome and ferrihemochrome complexes have a red pigmentation and have a visible spectrum similar to that of oxymyoglobin. However, the stability of the pigment is determined by rate of dissociation of the ligand bound to the sixth coordination position of the heme moiety. A ligand that dissociates quickly will oxidize causing
color fading or browning to occur (Livingston and Brown, 1981). Certain amino acids, denatured proteins, and nitrogen containing ligands can bind to the heme moiety once myoglobin has been denatured to produce a double band spectrum and often a pink color (Cornforth et al., 1986).

Nitrogen containing ligands can bind with the sixth coordination position of the heme moiety to produce a pink pigment known as nitrosohemochrome. The characteristic pink color of cured meat is due to nitrosohemochrome. This pigment is formed when nitrate, nitrite, and/or nitrous oxide are present in meat during cooking (Trout, 1989). The contamination of meat with nitrates, nitrite, and nitrous oxide will later be discussed in this paper.

Certain amino acids and denatured proteins can bind with the sixth coordination position of the heme moiety of denatured myoglobin to produce a pink pigment. The amino acids and denatured proteins that are able to bind with the heme moiety are produced by the degradation of endogenous meat proteins and non meat ingredients. Pyridine, histidine, cysteine, methionine, nicotinamide, or their side chains from the solubilized proteins and vitamin B₆ derivatives are the common ligands that bind with the heme moiety to produce a pink pigmentation in fully cooked poultry products (Ahn and Maurer, 1990a). In studies done by Cornforth et al. (1986), a complex of nicotinamide and heme pigment formed to generate a pink color in cooked turkey rolls. However, in studies done by Tappel (1957), a dihistidine complex was blamed for the occurrence of the pink color defect occurring in uncured, cooked turkey rolls, but Giddings (1977) disagreed with Tappel, saying that the second histidine bound to the sixth coordination position of the heme moiety was unstable and could easily be replaced by other ligands. Tarladgis (1962) suggested that a hemochrome whose fifth coordination position was bound to a carboxyl group and sixth coordination position was bound to water is responsible for the creation
of the pink pigmentation in uncured fully cooked meat. Various ligands were examined by many researchers to find a substitute for nitrite in cured meat products; the results discovered that histidine compounds and pyridine derivatives after reaction with the heme moiety would produce a pink pigment in fully cooked meat products (Akoyunoglou et al., 1963; Brown, 1973; Howard et al., 1973; Fox et al., 1974; Dymicky et al., 1975). Similar research was conducted by Ahn and Maurer (1990a), in which they found that pyridine and nicotinamide was the best heme complex forming ligands. The pyridine heme complex was the strongest when formed with myoglobin, hemoglobin, and cytochrome c. However, pyridine is not present in meat, but can be incorporated into a meat system through non meat ingredients. Trout (1989) suggested that the reaction between the heme moiety from myoglobin and nicotinamide present in the muscle was responsible for the pink color defect. The formation of the pink color was dependent on the concentration of nicotinamide in the muscle, which explains the sporadic occurrence of the pink color defect. Trout also stated that non meat ingredients used in precooked meat products cause color problems by altering the denaturation behavior of proteins. In another study completed by Ahn and Maurer (1990b), the most active heme complex forming ligand was nicotinamide. Ahn and Maurer (1990c) conducted experiments with turkey breast meat, in which pyridine, nicotinamide, and histidine were added. The samples with added pyridine, nicotinamide, and histidine exhibited significantly higher a* values (redness) when compared to controls. The higher a* values were due to heme complex formation by pyridine, nicotinamide, and histidine. Also in this study, cysteine, histidine, methionine, nicotinamide, pyridine, and added proteins formed heme complexes with many different meat pigments under denatured conditions. The pink color formed by the heme complex forming ligands faded rapidly upon exposure to air, which is a characteristic of the pink color defect. Ahn and Maurer stated that the amount of
heme complex forming ligands, such as histidine, nicotinamide, and solubilized proteins, meat pH, and the oxidation reduction potential are the most important factors in determining the color of cooked meat.

The meat pH plays a major role in determining the color of a meat product due to the amount of amino acids or proteins that are available for heme complex formation. As the pH of a meat system increases, the reactivity or the concentration of reactable amino acid side chains will increase due to the increase in electronegativity of the amino acid side chains from globin or other proteins. Also, at a higher pH the hydrogen ion dissociation from the amino acid side chains will increase (Ahn and Maurer, 1990b). The higher pH allows more available amino acids or proteins to be available for hemochrome formation, thus affecting the color of the meat. In studies done by Trout (1989), high pH muscle (pH > 6.0) from beef, pork, and turkey was redder in appearance than low pH muscle (pH = 5.5) and appeared undercooked. The suggested explanation for the pink color that formed was the amount of myoglobin denatured was reduced by the high pH. In this same study, the myoglobin in turkey breast exhibited minimum denaturation at pH levels between 6.50 and 7.00, except when cooking temperatures were high enough to completely denature myoglobin. The average pH of most meat is 5.4 – 5.8, but in meat from stress prone animals it is not uncommon for the pH to be as high as 6.50 or greater.

The oxidation reduction potential (ORP) plays a huge role in determining the color of a meat product. ORP is the extent to which a chemical ion exchanges electrons, which leads to electrical charges, during a chemical reaction. It is a non-specific measure that is similar to the measurement of pH. The ORP is an important factor in pink color formation because it determines the state of the hemochrome (Fe ++)/hemichrome (Fe ++++) iron of globin hemochromes. The ORP also controls the ability of pigments to combine with ligands. The
ORP is determined by the presence of reductants and is also a function of the pH of a meat product (Holownia et al., 2003). Cornforth et al. (1986) discovered that the presence of high reducing conditions allowed greater interconversion and reactivity of pigments that are undesirable from the point of meat color. Under reducing conditions, the pink color intensity of the heme complexes formed from myoglobin and hemoglobin with naturally present ligands increased immensely (Ahn and Maurer, 1990a). As the pH of a meat product increases, the ORP tends to become more negative (reducing conditions) (Holownia et al., 2003). There are a number of reductants that can be used in meat to reduce heme pigments, some of which are endogenous to muscle tissue. The presence of intracellular nicotinamide coenzymes is related to meat reducing activities, such as nicotinamide adenine dinucleotide phosphate (NADP+, reduced form NADPH) and nicotinamide adenine dinucleotide (NAD+, reduced form NADH) (Giddings 1974, 1977; Livingston and Brown 1981; Renerre 1990). In a study conducted by Ahn and Maurer (1989b), cooked ground turkey meat exhibited the normal chromatic absorption peaks of oxymyoglobin (542 nm and 580 nm) when the ORP was in the -15 mV to -100 mV range. When the redox potential was reduced to -550 mV the myoglobin was fully reduced to the ferrous state and only one chromatic absorption peak was observed at 556 nm. In a similar study conducted by Cornforth et al. (1986), the heme iron was found to be in the ferrous state when the ORP was -550 mV or less, resulting in formation of only pink reduced hemochromes. Ahn and Maurer (1989b) found that the addition of processing ingredients, such as salt and phosphate, will cause a change in the ORP of a meat product. The native redox potential of a meat product is in the range of +90 mV to -50 mV and changes in the ORP could have a strong effect on pinking in cooked turkey breast meat. Cornforth et al. (1986) offered a suggestion to prevent the pink color
defect in cooked meat under commercial conditions from occurring, it is necessary to maintain mildly oxidizing conditions.

Another major pigment found in the muscles of animals that has received blame for the formation of the pink color defect in uncured fully cooked poultry products is hemoglobin. Hemoglobin is the primary pigment of the blood, whose biological function is to distribute oxygen bound in the lungs throughout the body (Seideman et al., 1984). The majority of hemoglobin is removed from the body once the animal is stunned and bled, which makes the remaining myoglobin the main pigment associated with meat color. However, if an animal is improperly bled the remaining hemoglobin in the body of an animal is available for binding with ligands to form hemochromes similar to myoglobin.

Hemoglobin is a tetrameric, globular heme protein that has four hemes per molecule. The heme found in hemoglobin is the same as that found in myoglobin, which has six coordination positions. The sixth coordination position of heme moiety is available for reaction with ligands to possibly form hemochromes that will cause the appearance of the pink color defect. Hemoglobin would equally contribute to the color of a meat product along with myoglobin, if hemoglobin was found in the same concentration.

Cytochrome c is another potential cause of formation of the pink color defect being found in uncured fully cooked poultry white meat. Cytochrome c is an electron transport protein present in all organisms having mitochondrial respiratory chains (Fleming et al., 1991; Holownia et al., 2003). The residual pink color being found in poultry white meat is thought to be a result of cytochrome c heat stability. Unlike myoglobin, cytochrome c remains stable at high temperatures that denature myoglobin. Cytochrome c has been found to withstand denaturation
temperatures as high as 105º C, which makes it extremely heat stable (Cornish and Froning, 1974).

The pH of a muscle will affect the amount of cytochrome c present in that particular muscle. In research done by Pikul et al., (1986), muscle with a high pH (approximately 6.6) contained a higher concentration of cytochrome c when compared to muscle with a lower pH (approximately 6.0 to 6.1). However, in research done by Ahn and Maurer (1990b), the pH did not greatly affect the heme complex forming reactions of cytochrome c. The heme complexes formed between cytochrome c and selected ligands were more stable than those formed with myoglobin and hemoglobin. The cytochrome c content of chicken meat may also be influenced by the type of chilling method (ice slush against air chill) used to chill the carcass. In this research, the cytochrome c content for air chilled (27.15 mg/g) birds was double that of ice slush (13.71 mg/g), but no explanation was given by the authors (Fleming et al., 1991). The pinkness found in fully cooked uncured breast meat could be caused by cytochrome c instead of myoglobin, but the amount of myoglobin found in the muscle is 50 fold higher than cytochrome c in turkey breast muscle (Ahn and Maurer, 1989a, b).

2.5 Non Meat Ingredients and Cooking Methods

The pink color defect that has plagued the poultry industry can be caused by the use of non meat ingredient in further processed products. The market for poultry products has shifted over the years to be predominately further processed products. The consumers in the marketplace are shopping for convenience items and the further processed poultry products being produced are meeting these demands. However, to meet the demand created by consumers, processors have to continuously create new products, which involve the use of non meat
ingredients. These ingredients being used help to improve the functionality of the product by increasing marination pickups and providing a wide variety of different flavors.

The main two ingredients used by processors to increase marination pickups are salt (NaCl) and phosphates. These two ingredients enter the meat tissue and bind with the actin and myosin proteins within muscle, which creates binding sites for water. The water molecules will bind with the salt and phosphate to become bound within the muscle, which will increase the marination pickup as well as, from a consumer aspect, increase the juiciness and flavor of the product.

However, a well known oxidizer of myoglobin is salt. There are two mechanisms by which salt promotes the oxidation of myoglobin. Lowering the buffering capacity of meat is the first mechanism. The dissociation of oxygen from the heme moiety, which oxidizes myoglobin to metmyoglobin, is a result of denaturation caused by low pH. Acids are oxidizing agents that will cause oxidation of myoglobin to occur, especially if the buffering capacity of the meat has been reduced by the addition of salt. A decrease in the uptake of oxygen is the second mechanism by which salt promotes the oxidation of myoglobin. The decrease in uptake of oxygen will cause low oxygen tensions within the meat resulting in oxidation of myoglobin (Seideman et al., 1984).

The use of additives such as salt and sodium tripolyphosphate (Na₅P₃O₁₀) may cause color problems in turkey breast because these additives alter the denaturation behavior of proteins such as myoglobin (Trout, 1989). In research done by Trout (1989), he discovered that the percentage of myoglobin denatured increased linearly with increasing sodium chloride concentrations. Trout also concluded that salt will decrease the pinkness of cooked meat products. In the same experiment, Trout found that sodium tripolyphosphate increased the
amount of denatured myoglobin, but it increased the pinkness of cooked meat products. Trout suggested that the reason for the pink defect appearing was because of the increase in pH produced by the sodium tripolyphosphate. However, Trout stated that the tripolyphosphate ion reduces the pinkness of cooked meat products and recommended a neutral blend of phosphates, such as a mixture of sodium tripolyphosphate and sodium acid pyrophosphate, be used to inhibit the increase in pH caused by phosphates. In research completed by Ahn and Maurer (1990b), added salt solubilized myofibrillar proteins and increased the number of exposed reactable side chains, which increased the chance for heme complex formation. Also, this increases the chance for occurrence of the pink color defect, which may depend upon which heme complex was formed from the solubilized proteins and exposed side chains.

Increases in water holding capacity and protein solubility make salt a commonly used additive in heat treated meat products. Salt accomplishes this task by increasing the ionic strength of the meat product, which allows more proteins to be solubilized. The ionic strength of a meat product is the amount of dissociated ions available in a meat product. The temperature at which denaturation of myoglobin occurs is influenced by the ionic strength of a meat product. In research done by Kristensen and Purslow (2001) showed that the heme molecule was protected from heat induced destruction by the addition of salt. The meat products produced using salt had greater amounts of heme iron than meat products produced without salt. Also, the meat products produced with added salt showed decreases in cooking loss as compared to the meat products produced without salt addition. The authors suggested that the increase in ionic strength provided the protecting effect on the heme molecule by influencing the rate of chemical reactions.
The ionic strength of a meat product is continuously changing from the time the animal is harvested to the table of a consumer. In studies done by Feidt and Brun-Bellut (1999), the ionic strength of a meat product begins to increase during the development of rigor mortis due to ion release. Ions are released by lactate and organic acids during anaerobic glycolysis. The ionic strength of a meat product was not affected by chilling treatments. The authors concluded that ionic strength plays a major a function in post mortem muscle changes.

The pH of a meat product can influence the ionic strength. As the pH of a meat product decreases, the amount of available ions increases (Feidt and Brun-Bellut, 1999). In an experiment by Gil et al. (1998), the authors discovered that protein solubility of different types of meat, including poultry, increased in accordance with the increasing ionic strength. The solubility of the myofibrillar protein extracts accounted for most of the difference found. The characteristics of the muscle and the myofibrillar protein solubility are modified by changes in the salt concentration or in the pH of meat (Dilber-Van Griethuysen and Knight, 1991). However, the protein extractability is highly dependent upon the ionic strength and pH of the salt solution used on the meat product (Franks 1993).

The water holding capacity of muscle and muscle products are influenced by salts and pH. Improvement in physical properties of products and increased profits are a result of increased water uptake and water holding capacity of muscle protein gels, therefore, this becomes an important issue for processors. The results from studies done by Kristinsson and Hultin (2003) have shown that increases in pH cause myofibrils to expand. The expansion of the myofibrils will allow more water to be taken up and less to be lost. It is common belief that only under high salt conditions that proper gelation can be achieved because of myofibrillar protein solubility, but Kristinsson and Hultin (2003), have shown that strong gels with excellent water
holding capacity can be formed under low ionic strength conditions. The myofibrillar proteins were essentially insoluble under these low salt conditions when the gels were formed. The authors also demonstrated that the gels formed at pH 7.0 with low ionic strength had greater gelation and water holding capacity than the gels formed at lower pH values. The authors stated that less water was taken up by chicken breast when the ionic strength was increased because the addition of salt caused a decrease in filament spacing and that the electrostatic repulsion within the cell matrix was lowered. The authors suggested the theory that the driving force for water uptake is repulsion created by the negative charges of the proteins. In research completed by Feng and Hultin (2001), chicken breast muscle proteins formed stronger, more evenly distributed gels at pH 7.0 with low ionic strength than at pH 6.4 with low ionic strength.

The use of phosphates as an additive in meat products is a common practice among processors to increase water holding capacity and tenderness of the products. There are many different types of phosphates being used in meat products, which include the most commonly used sodium tripolyphosphate along with sodium acid pyrophosphate and sodium orthophosphate. There has been an increase in the use of inorganic, alkaline sodium phosphates in meat products of beef, chicken, and pork due to their beneficial effects. The use of sodium phosphates improves functionality, palatability, and storage stability of meat products. Sausages and restructured meat product processors are using polyphosphates to enhance water holding capacity, color, flavor, and texture, which in turn improves quality of the products (Lee et al., 1998). The results of a study done by McGee et al. (2003) indicated that the use of sodium tripolyphosphate increased tenderness and sensory characteristics as well as decreased cooking loss and lipid oxidation.
During the storage of meat and precooked meat products, lipid oxidation can cause rancidity and off flavor problems. Phosphates are used to bind prooxidants in a meat product and reduce off flavor as determined by a trained sensory panel. Also, the pH and ionic strength of meat products are increased with the addition of phosphates (Cheng and Ockerman, 2003). The increase in pH of a meat product by phosphates improves water holding capacity (Allen et al., 1998). Phosphates improve the functional properties of PSE meat by increasing the pH and ionic strength. In research done by Torley et al. (2000), cook loss of PSE meat was reduced by polyphosphate when compared to PSE meat without polyphosphate addition. The same results were found when normal muscle was used in the place of PSE muscle. However, during this experiment, less protein was extracted from the PSE muscle when compared to the normal muscle when a combination of pyrophosphate and salt was added. Also, a large percentage of the PSE muscle myofibrils did not swell, whereas, all of the normal muscle myofibrils swelled equally. The myofibrils of the PSE muscle did not swell equally because some of the myosin heads were partially denatured, which prevents the actomyosin from being split by the polyphosphate and limits swelling. The authors also discovered that the use of polyphosphates in a high ionic strength solution will cause the non-denatured PSE muscle myofibrils to swell and produce a three dimensional protein network that will bind water and retain it after heating. However, the addition of polyphosphates will only improve the functionality of non-denatured PSE myofibrillar, but will not improve the denatured muscle proteins.

The use of added phosphates can produce color problems in products, which can be linked to the occurrence of the pink color defect in uncured, fully cooked poultry products. In research done by Young and Lyon (1994), turkey breast muscle color was altered due to the increase in pH caused by the addition of sodium tripolyphosphate prior to the resolution of rigor.
In this experiment, the CIE a* value or redness of the turkey breast meat was increased by the addition of sodium tripolyphosphate. The authors suggest that the increased pH inhibited myoglobin denaturation. The sensitivity of myoglobin to heat denaturation was shown in model systems to decrease as muscle pH increased (Trout 1989). However, in studies done by Young et al. (1996a), the addition of sodium tripolyphosphate to postrigor muscle decrease the CIE a* values in cooked turkey breast meat. In comparison of the studies completed by Young and Lyon (1994) and Young et al. (1996a), the addition of phosphates to prerigor muscle increased the pink color of the uncured, fully cooked turkey breast meat; whereas, addition of phosphates postrigor decreased the pinkness of the uncured, fully cooked turkey breast meat. The reason being the myoglobin in the postrigor muscle was already denatured due to the decrease in pH during rigor mortis. The addition of phosphate to prerigor muscle kept the pH high providing a protective effect for myoglobin, which produced a product with the pink color defect due to undenatured myoglobin.

Another benefit of phosphate addition comes from a microbial aspect. In a study done by Pathgeber and Waldroup (1995), the microbial population of poultry meat was altered by chilling the chicken carcasses in commercial blends of polyphosphates. The use of trisodium phosphate (TSP) is an effective antimicrobial agent in controlling Campylobacter, E. coli, L. monocytogenes, Salmonella, S. aureus, and psychrotrophs. Many hypotheses have been made to explain the antibacterial mechanism through which TSP reduces microorganisms, but it is not fully understood. Some researchers have speculated that a thin layer of fat and bacteria are removed from the surface of poultry skin by TSP. Others have suggested that TSP chelates essential metal ions in the cell walls, which reduces the number of microorganisms (Vareltzis et al., 1997). In a study completed by Vareltzis et al. (1997), the use of sodium tripolyphosphate
reduced the amount of spoilage bacteria on poultry carcasses. The carcasses treated with sodium tripolyphosphate exhibited less slime and off odors when compared to controls not treated with sodium tripolyphosphate. Also, the treated carcasses had three extra days of shelf life when stored at 4°C when compared to the untreated carcasses.

Another non meat ingredient commonly used in meat products that has similar microbial reduction capabilities is sodium citrate. Sodium citrate uses a combined effect of cation chelation and mineral deprivation of the bacteria to provide microbial reduction (Miller et al., 1993). In studies done by Long and Phillips (2003), the authors concluded that sodium citrate provided a multiple hurdle approach at controlling populations of spoilage and pathogenic bacteria on, or in, meat or meat products.

In meat products, sodium citrate is commonly used in combination with phosphate and in some cases takes the place of phosphate in phosphate free meat products. Sodium citrate is used to improve the texture and water holding capacity of meat products by raising the pH (Ruusunen et al., 2003). Sodium citrate has recently received attention by researchers as a possible inhibitor of the pink color defect being found in uncured, fully cooked poultry products because it is a chelator that has the potential to bind the heme iron of myoglobin. This prevents other ligands from binding to the heme that could possibly cause formation of the pink color defect (Sammel and Claus, 2003a). In a study done by Sammel and Claus (2003a), sodium citrate did not have any affect on the pinkness of intact turkey breast that were treated with sodium nitrite and nicotinamide, which are pink inducing agents. However, sodium citrate effectively reduced pinking in ground turkey meat that was treated with both pink inducing agents, especially nicotinamide. The authors suggested that the pinkness of the intact turkey breast was not
reduced by sodium citrate because it was unable to access the heme of myoglobin and prevent
the pink color generating ligands from binding.

Citric acid is also a chelator that has similar effects as sodium citrate. In an experiment
conducted by Kieffer et al. (2000), citric acid inhibited the pink color defect from forming in
ground turkey meat. In a study done by Sammel and Claus (2003a), citric acid used at 0.2% and
0.3% (meat weight basis) was found to effectively reduce pinking in ground turkey meat treated
with known pink inducing agents, sodium nitrite and nicotinamide. In the same study, citric acid
used at 0.1%, 0.2%, 0.3%, and 1.0% (meat weight basis) had no effect on pinking of intact
turkey breast treated with sodium nitrite and nicotinamide. The authors suggested that the
pinkness of the intact turkey breast was not reduced by citric acid because it was unable to access
the heme of myoglobin and prevent the pink color generating ligands from binding. In another
study researched by Yang and Chen (1993), chicken breast marinated with citric acid had
reportedly decreased external and internal CIE a* values (redness). The disagreement between
the previous two studies can possibly be contributed to the use of turkey rather than chicken.

There is an assortment of other ingredient to go along with sodium citrate and citric acid
that can reduce pink color formation in cooked poultry products. Ethylenedinitrilo-tetraacetic
acid disodium salt, diethylenetriamine pentaacetic acid, and trans 1,2-diaminocyclohexane-
N,N,N’,N’ tetraacetic acid monohydrate were shown by Schwarz et al. (1997) to reduce pink
color. However, none of the above pink reducing ingredients can be legally added to meat.
Whey protein concentrates and nonfat dried milk has exhibited a capability for reducing pink
color in meat products by interacting with pink generating ligands inhibiting their ability to
produce pink color (Sammel and Claus, 2003a; Sammel and Claus, 2003b). Also, these two
ingredients are approved for use in meat products (Sammel and Claus, 2003a).
In research done by Schwarz et al. (1997), nonfat dried milk was found to reduce pinking of meat products that were contaminated by known pink inducing agents, sodium nitrite and nicotinamide. The ability of nonfat dried milk to reduce pink color in poultry products can be related to an increase in the oxidation reduction potential of the product, which leads to a reduction in formation of pink generating hemochromes (Dobson and Cornforth, 1992). In a study researched by Slesinski et al. (2000), whey protein concentrates were found to reduce pink color in turkey products that were caused by nicotinamide or nitrite contamination. However, in research done by Dobson and Cornforth (1992), whey protein concentrates were found to increase the pink color in uncured, fully cooked turkey rolls. In another experiment conducted by Sammel and Claus (2003b), whey protein concentrates were found to effectively reduce the pink color generated by nicotinamide in ground turkey breast, but was unsuccessful in reducing pinking due to sodium nitrite. The authors suggest that the whey protein concentrates were not effective in reducing pinking caused by sodium nitrite because the nitrite had already reacted with myoglobin before the unfolding and interaction of the whey protein concentrates, where as, the nicotinamide hemochrome could not form until heating of the product, which allowed time for the whey protein concentrates to unfold and expose reactive side chains. When added to meat, whey proteins are in their normal globular conformation and heating is required to unfold and expose the amino acids making them available for interaction with myoglobin (Rockell, 1989).

The end point cooking temperature is another industry procedure that has received attention as a possible combatant of the pink color defect being found in uncured, fully cooked poultry products. In research done by Helmke and Froning (1971), they stated that the pinkness of poultry meat increased when end point cooking temperatures were below 71°C because
myoglobin remains only partially denatured after cooking. Nevertheless, end point cooking temperatures in poultry further processing facilities should never drop below 71°C because 71.1°C is the lowest end point cooking temperature allowable according to USDA regulatory limits (Claus et al., 1994). In a study done by Davis and Franks (1995), it was found that the pinkness of poultry meat decreased as the end point cooking temperature was increased. The authors also found that the percent denatured myoglobin increased as the end point cooking temperature was increased. Similar results were found in a study done by Girard et al. (1989), the pinkness of uncured poultry meat decreased as the end point cooking temperature increased with the most denatured myoglobin occurring at the end point cooking temperature of 85°C. The incomplete denaturation of myoglobin at low end point cooking temperatures is the reason given by Trout (1989) for the formation of pink color in uncured, fully cooked high pH poultry meat products. However, research done by Claus et al. (1994) disagrees with the above researchers. Claus suggest that lower end point cooking temperatures will reduce the incidence and severity of the pink color defect being found in poultry products because at higher end point cooking temperatures more myoglobin is denatured and available for reaction with pink color generation ligands, such as nicotinamide.

2.6 Nitrate/Nitrite Contamination

One sure way for a meat product to contract the pink color defect is by nitrate/nitrite contamination. Nitrates/Nitrites present in water being used in poultry processing plants can be a cause of the “pinking” or pink color defect found in poultry meat. This is a major problem that causes economic loss and customer dissatisfaction in uncured fully cooked poultry products. The contamination of meat with nitrites or nitric oxide from a wide variety of sources is the most widely accepted explanation. Injectors, smokehouses, stuffers, other processing equipment,
water supply, and the diet of the birds are possible sources of nitrate or nitrite contamination of meat (Cornforth et al., 1986). The drinking water and feed of birds may lead to contamination with nitrates and nitrites as well as a pink end product (Froning et al., 1967). Nitrogenous contamination can also originate from previously used processing equipment for curing and from water utilized for carcass chilling (Mugler et al., 1970). The previous research done has concluded that the quality of processing water used in poultry plants from chilling to marinade formulation could cause the pink color defect to occur in uncured, fully cooked poultry products.

Nitrites are a widely used additive in food and food systems, especially in the curing of meats. In cured meats, sodium nitrite is allowable up to levels of 156 ppm in the United States. Sodium nitrite is the precursor to the formation of the pink cured meat pigment, mononitrosylhemochrome (Killday et al., 1988). Nitrite provides the characteristic pink color and flavor in cured meats and acts as an effective antibotulinal agent and preservative (Nakamura and Nakamura, 1996). In the curing of meat, nitrite has at least three functions. Firstly, mononitrosylhemochrome is produced by a reaction of nitrite with myoglobin to provide the characteristic pink color of cured meat. Secondly, nitrite contributes to the flavor profile of cured meat by inhibiting the development of rancid off-flavors. Also, nitrite prevents the growth of spoilage bacteria in the meat and most importantly Clostridium botulinum. Nitrates are also used in the curing of meats. Nitrate (NO₃) is broken down and reduced to nitrite (NO₂) by bacteria in anaerobic conditions, using the molybdopterin-containing nitrate reductase. Also, bacteria in the mouth and stomach can reduce dietary nitrate to nitrite.

The safety of nitrites in the diet is a concern. In high concentrations, nitrite is toxic to humans. Oxidation of oxyhemoglobin to ferrihemoglobin which leads to methemoglobinemia is the main toxic effect. This can cause “blue baby syndrome” in newborn infants because the
methemoglobin reducing capacity is low and can be fatal. There are a number of different sources of nitrate and nitrite in the diet. Vegetables are a major source along with contaminated drinking water, medicines, and sausages. For example, root vegetables such as potatoes contain 200 mg/kg and leaf vegetables such as lettuce contain 1000 mg/kg of nitrate. Also, there has been a possible link between nitrite and cancer established since the 1970s. In a wide range of animal species, N-nitroso compounds (nitrosamines) have been shown to be carcinogenic and have been detected in cured meats after cooking (Cammack et al., 1999).

One major source of nitrates and nitrite found in water and vegetables is coming from surface leaching and run-off from farm applications. The amount of fertilizers, pesticides and imported animal feedstuffs being used is increasing to meet the demands for food production and to get high yielding crop varieties. The amount of Nitrogen containing fertilizers being using in the UK has increased sixfold since the 1950s. The main cause of surface water and ground water degradation over the years can be contributed to excessive loss of nutrients from the soil as well as farm effluents in surface runoff and leaching (Hooda et al., 2000). Ground water discharges and sub-surface flow including tile or pipe drainage into streams from draining grassland and arable areas have contributed to large concentrations of nitrates being found in streams and other watercourses (Baker and Laflen, 1983; Hallberg, 1987; Gangbaz et al., 1995). The actual amount of nitrate in surface runoff is minimal, but large amounts of nitrogen contain compounds in runoff through nitrification and mineralization contributes to the total amount of nitrates found in ground water and water sources. The surface waters draining from arable farmland is usually significantly higher in nitrate concentrations than the runoff from livestock farming areas. This can be contributed to the nitrogen containing fertilizer used and to the nitrification and soil mineralization processes that occur during the plowing process after each crop or manure
application. The intensity of agricultural production usually reflects on the amount of nitrate concentration found in that particular area.

There are two forms of inorganic nitrogen in the soil, which are nitrate and ammonium. As water moves through the soil, it is able to pick up nitrate ions because they are freely mobile below the rooting zone. The land use and management practices applied influence the extent of nitrate leaching. Also, the type of soil and its texture play an important role in the amount of leaching of nitrates that can occur (Hooda et al., 2000). The largest losses and greatest amount of leaching occurs in sandy and peat soils because of its loosely bound texture as compared to clay soil, which has a tighter packed texture and the smallest losses and least amount of nitrate leaching (Bergstrom and Johansson, 1991). The time of manure and fertilizer application can significantly increase the concentration of nitrates found in the soil and impact the amount of nitrates leaching into ground water. This is contributed to the amount of nitrogen the plant needs at the time of application. Plants require the most nitrogen during the spring months because it is the growing season and the nitrogen is pulled up by the roots and utilized for growth and energy by the plant. During the autumn and winter months the plants do not require as much nitrogen because they are not in a growth mode. The amount of manure and fertilizer applied to crops should be adjusted to meet the requirements of the plant to cut down on the amount of excess nitrogen introduced into the soil for leaching into ground water and to reduce the economical impact for the farmer (Bailey, 1993). August or September application of manure resulted in smaller nitrate losses when compared to an October or November application in research done by Froment et al. (1992) and Smith and Chambers (1993).

As more and more nitrates and nitrites are being found in ground water and in water sources, poultry plants need to be aware of the quality of water being used in processing.
applications as well as sanitary applications. Ahn and Maurer (1989a), reported that as little as 1 ppm nitrite ion causes pinking in turkey breast meat. Also, in sensory panels conducted by Heaton et al. (2000), pinkness was detected by trained panelist for pork shoulder, turkey breast, and chicken breast at sodium nitrite levels of 4 ppm, 2 ppm, and 1 ppm, respectively. This research has concluded that low levels of nitrite contamination is responsible for the pink color defect being found in uncured fully cooked poultry products. The use of water filtration can be applied to the incoming water source to improve the overall water quality and reduce the amount of nitrates and nitrites. The use of reverse osmosis filtration has been very effectively applied to water denitrification as shown by Schoeman and Steyn (2003). In their research the nitrate concentration was reduced from 42.5 mg/l in the influent stream of the reverse osmosis filtration system to 0.9 mg/l in the permeate water.

2.7 Irradiation

One processing preservation technique that is increasing in popularity and has recently been approved for use on poultry products is irradiation. Irradiation can have a negative effect on meat color by causing formation of the dreaded pink color defect, but is an excellent method to improve the microbial safety of meat (Nam and Ahn, 2002a). Consumers have easily misconstrued the pink color produced by irradiation to be the color of an undercooked product and have linked the product and processor to poor quality (Du et al., 2002).

The color and odor of meat products are affected by exposure to irradiation. The ultimate acceptance of a meat product by consumers is determined by the color and odor of the product. The sulfur compounds produced by the irradiation process were related to the off odors detected in irradiated meat (Ahn et al., 2000). The production of carbon monoxide during irradiation was the suggested cause of color changes induced in irradiated meat (Millar et al., 2000).
In research done by Nanke et al. (1998), the formation of an oxymyoglobin pigment was responsible for the pink color produced in irradiated products. However, in research done by Millar et al. (1995), the red/pink color produced in irradiated meat products was caused by a ferrous myoglobin derivative, such as carboxymyoglobin or nitric oxide myoglobin, rather than oxymyoglobin. The irradiation of raw poultry products was found to reduce the oxidation reduction potential as well as produce a gas compounds that can bind with myoglobin as the sixth ligand (Nam and Ahn, 2002b).

The oxidation reduction potential of broiler breast fillets was significantly decreased by irradiation, in research done by Du et al., (2002). The author suggested that the decrease in oxidation reduction potential was due to the electrons absorbed into the meat during irradiation with an electron beam. However, after 3 and 7 days of storage, the oxidation reduction potential of the irradiated breast fillets that were aerobically packaged was significantly higher than that of non irradiated breast fillets. The authors suggested that the accelerated changes in oxidation reduction potential of the irradiated breast fillets was related to the increase in oxygen permeability through the membranes due to damage induced by irradiation. However, in the same study, the oxidation reduction potential remained low for the irradiated breast fillets packaged under a vacuum. In research completed by Nam and Ahn (2002b), the oxidation reduction potential of aerobically and vacuum packaged poultry meat was decreased by irradiation, but the oxidation reduction potential of the aerobically packaged meat rose to be higher than that of non irradiated meat after storage.

The reduced conditions of irradiated poultry meat caused the iron of myoglobin to be reduced to ferrous iron, which has a strong affinity to accept a ligand and produce a red color. Hemochrome formation is promoted by reducing conditions and prevented by oxidizing
conditions (Cornforth et al., 1986). In research done by Millar et al. (2000), the CIE a* value (redness/pinkness) of chicken breast fillets were increased by irradiation. The color of meat is influenced by any condition, such as irradiation, that can change the oxidative status of the heme iron and produce a ligand compound that can easily bind to the sixth coordination position of the ferrous iron heme moiety (Nam and Ahn, 2002b). The irradiation process produces carbon containing gases, such as CO₂, CH₄, and CO. The pink color formed in poultry meat due to irradiation is believed to be caused by carbon monoxide (CO) because it is a strong field ligand that is able to form complexes with the heme pigments, as well as, bind as the sixth ligand to myoglobin (Nam and Ahn, 2002a).

In a study conducted by Du et al. (2002), the CIE a* value (redness) of irradiated meat was increased by irradiation due to the production of CO and reduced oxidation reduction potential. When non irradiated meat was exposed to the same concentration of CO, the color of the meat was not affected. The reduced oxidation reduction potential allowed the CO to form complexes with the heme pigments and/or become the sixth ligand of myoglobin to produce the pink color defect in irradiated meat products. However, in the same study, the pinkness of the irradiated meat faded after 3 days of aerobic storage and was not statistically significant from the non irradiated meat, but the vacuum packaged irradiated meat retained it pink color throughout storage. The authors suggested that the decrease in pinkness of the aerobically packaged irradiated meat is due to evaporation of the carbon monoxide. Also, the authors suggest that aerobic display of irradiated meat is a good method for preventing the pink color defect in this type of product.
2.8 Conclusion

In conclusion, the research done over the years attributed the pink color defect to be caused by (1) preslaughter factors such as heat and cold stress, gaseous environment, genetics, feed, hauling, and handling, (2) stunning techniques, (3) various classes and types of pigments, (4) current industry procedures including the use of nonmeat ingredients and cooking methods, (5) incidental nitrate/nitrite contamination through water supply, diet, freezing and processing equipment, and processing ingredients, and (6) irradiation of precooked products. The in situ conditions of the meat products being produced are represented by the oxidation reduction potential, pH, degree of denaturation of myoglobin, and the presences of reactive ligands in combination with the factors listed above are related to formation of the pink color defect being found in uncured fully cooked poultry products (Holownia et al., 2003). A greater understanding of the factors that cause the pink color defect and the endogenous conditions of the meat by the processor may help to reduce the occurrence of the pink color defect. The use of good manufacturing practices in combination with mildly oxidizing conditions and non meat ingredients that reduce pink color formation in the production of further processed poultry products should produce a quality product free of the pink color defect.

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CHAPTER 3

THE PREVENTION AND PREDICTABILITY OF

PINK COLOR FORMATION IN

UNCURED FULLY COOKED CHICKEN BREAST MEAT

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1 Wills, C.T., A.E. Reynolds, R. Toledo, L. Wicker. To be submitted to *Journal of Food Science*. 
3.1 Abstract

The occurrence of the pink color defect in poultry white meat results in serious economic losses to processors because of the association with undercooking by the consumer. The use of marinades formulated with sodium citrate, citric acid, and various phosphates with a controlled ionic strength may reduce the occurrence of the pink color defect. A pinking prediction equation was established as a function of the pH and oxidation reduction potential (ORP) of individual cooked chicken breast to determine the probability of pink color formation within the breast. However, pinking may be related to a combination threshold for the individual cooked muscle pH and cooked ORP that needs further research to be directly pinpointed. A method to control the pink color defect would be to sort raw chicken breast by color, to tailor marinade formulation to minimize the probability of pink color formation and maintain yield and quality.

INDEX WORDS  Pink color defect, pink color, marination, pH, oxidation reduction potential, Ionic strength, Predictability, Yield
3.2 Introduction

One of the first impressions that consumers have about a meat product is color. The color of a meat product is the easiest quality trait to evaluate and the most utilized by consumers (Schwarz et al., 1997). The initial selection in the marketplace of a raw meat product by a consumer and the final acceptance of the cooked product upon consumption is affected by color of the initial product (Fletcher, 1999). The color of products produced by the meat industry is understood by the consumer to reflect quality of that product. Products with color variation or off colors are perceived by the consumer to be low quality and even possibly the result of spoilage or undercooking. A major problem that has plagued the meat industry, especially the poultry industry, is the occurrence of a pink color defected in uncured fully cooked meat and poultry products (Schwarz et al., 1997).

The pink color defect can be described as the development of a pink color or tinge in cooked poultry white meat that has been cooked to an internal temperature far above that required by United States Department of Agriculture (USDA), which gives the appearance of an undercooked product and leads to consumer rejection. The occurrence of this defect is sporadic in a variety of poultry products such as uncured fully cooked chicken breast and in uncured turkey and pork rolls. The exposure to air causes a rapid fading of the pink pigment, but the appearance of an unsafe, undercooked product still causes consumer complaints and commercial buyer discounting (Schwarz et al., 1997). This defect has been the subject of many years of research and has resulted in serious economic losses to processor, producers, and retailers of poultry products.

The cause of the pink color defect in uncured fully cooked poultry products has been attributed to various factors. The research done over the years has attributed the pink color
defect to be caused by (1) preslaughter factors such as heat and cold stress, gaseous environment, genetics, feed, hauling, and handling (Froning and Hartung, 1967; Froning et al., 1968a, 1969a, 1978; Babji et al., 1982; Ngoka et al., 1982; Sackett et al., 1986), (2) stunning techniques (Ngoka and Froning 1982; Froning, 1995; Young et al., 1996a; Craig et al., 1999), (3) various classes and types of pigments (Fox, 1966; Tappel, 1957; Ledward, 1974; Livingston and Brown, 1981; Izumi et al., 1982; Cornforth et al., 1986; Girard et al., 1989; Ghorpade and Cornforth, 1993; Smith, 1998), (4) current industry procedures including the use of nonmeat ingredients and cooking methods (Pool, 1956; Froning et al., 1968c; Helmke and Froning, 1971; Janky and Froning, 1973, Trout, 1989; Ahn and Maurer, 1989a, 1990a,b; Claus et al., 1994; Cornforth et al., 1998), (5) incidental nitrate/nitrite contamination through water supply, diet, freezing and processing equipment, and processing ingredients (Froning et al., 1968b, 1969b; Mugler et al., 1970; Nash et al., 1985; Ahn and Maurer, 1987; Fleming et al., 1991; Heaton et al., 2000), and (6) irradiation of precooked products (Nam and Ahn, 2002a,b). The in situ conditions of the meat, such as pH, degree of denaturation, reactivity of endogenous meat compounds and reducing conditions are all affected by the previously identified factors. The changes in the in situ conditions of the meat change the structure, chemical state, and reactivity of the pigments found in the meat (Holownia et al., 2003). However, the color of meat changes along with the changes produced by the factors discussed above. The color of meat is attributed to the light-scattering matrix of cellular material, myofibrillar proteins, connective tissue, and light-absorbing pigments (MacDougall, 1970; Swatland, 1983). The light scattering and absorbing properties of meat are affected by changes in the in situ conditions of the product (Holownia et al., 2003). Therefore, the final color of raw meat products is determined by the changes of in situ conditions that are caused by many intrinsic and extrinsic factors.
Preslaughter conditions that animals are exposed to during growth and shipment to processing plants can have adverse effects on the quality of meat produced from that animal. Harsh conditions that induce stress upon the animal during growth, transportation, and holding, such as periods of high, low, or wide temperature fluctuations, gaseous environment, feed and water deprivation, hauling, and handling, will cause a low quality meat to be produced. The pale, soft, and exudative (PSE) condition caused by low meat pH and the pink color defect caused by high meat pH are the best examples of stress effects on muscle quality (Wood and Richards, 1975).

In studies done by Wismer-Pedersen (1959) and Forest et al. (1963), periods of wide temperature fluctuations or high temperatures increased the occurrence of the PSE condition in pork. Pigs held at elevated temperatures immediately before slaughter experienced a rapid rate of postmortem muscle glycolysis as indicated by a rapid decline in muscle pH and decreased muscle color intensity (Sayre et al., 1963). Dark cutting meat and high pH were exhibited in cattle exposed to low winter temperatures prior to slaughter (Lawrie, 1966). In chicken broiler muscle, Wood and Richards (1975) observed cold stress lengthened the period of postmortem glycolysis while heat stress hastened the rate of postmortem glycolysis. Similar results were found by Froning et al. (1978) and Lee et al. (1976), in which preslaughter heat stressed birds produced a lower pH than birds observed under cold stress conditions. Also, in experiments done by Babji et al. (1982), breast meat from heat stressed birds had significantly lower raw and cooked pH values, lower water holding capacity, higher cooking loss, and was less desirable than the breast meat from the control or cold stressed birds. In experiments conducted by Froning et al. (1978) significantly lower L* (darker) and higher a* (redder) values were found in white meat from heat stressed birds. Prolonged exercise raised the pH value and juiciness of meat was
reported by Briskey et al. (1959). The pH of the meat is greatly affected by exposure to stressful environments such as high and low temperatures.

Poultry meat color is markedly affected by the final pH of meat after slaughter. Meat with low pH values (pH < 5.7) exhibit a pale, less red color where as higher pH meat (pH > 6.3) gives a darker, redder color (Babji et al., 1982). Muscle pH and meat color are highly correlated as shown by previous research. Breasts with low muscle pH are commonly observed to have a light color, where as breast muscles with a high pH are shown to be dark in color (Fletcher, 1999). The final pH of the breast muscle is directly related to the biochemical state of the breast at the time of slaughter and during rigor mortis development. The light reflectance property of the breast is influenced by the pH. The biochemical state of the breast muscle is determined by the rate of postmortem glycolysis. An increase in the rate of postmortem glycolysis will cause a rapid pH decline and will cause the breast to have a light pale color (Babji et al., 1982). The pH decline is a product of the lactic acid produced during postmortem glycolysis. As birds are held at higher temperatures prior to slaughter the rate of postmortem glycolysis will increase to produce meat with pale color and low pH (Wood and Richards, 1975). Cold stress prior to slaughter will reduce the rate of postmortem glycolysis and produce meat with a darker color and higher pH. The changes in pH are all due the conditions the bird experienced prior to being slaughtered. The importance of proper procedures preslaughter is essential in producing a high quality product that has normal color and functionality.

The types of gases birds are exposed to during growth and preslaughter procedures could be another cause of the pink color formation occurring in fully cooked poultry products. Hydrogen sulfide, methane, ammonia, carbon monoxide, and carbon dioxide are the predominant gases found in poultry houses during growth of birds (Day et al., 1965; Taeginides and White,
1969; Ludington et al., 1971). Ammonia is produced in houses mainly from the excretion of the birds. The presences of high concentrations of ammonia within poultry houses significantly increased the amount of carcass condemnations due to undergrades and breast blisters (Quarles and Kling, 1974). In research done by Sackett et al. (1986), the presence high concentration of ammonia (75 ppm and 100 ppm) caused birds to become more restless and excited than birds not exposed to ammonia. However, the exposure to ammonia did not have an effect on the color or pigment concentration in muscle.

Carbon dioxide is present in normal atmospheric air at 300 ppm and is an odorless gas (Handbook of Chemistry and Physics, 1978). Carbon dioxide is predominantly produced by respiration of the birds in enclosed houses. In poultry houses, carbon dioxide concentrations range from 300 to 2,500 ppm during the winter and 400 to 1,300 ppm during the summer months (Nakaue et al., 1981). High concentrations of carbon dioxide may cause stress due to asphyxiation and deprivation of oxygen. In research conducted by Sackett et al. (1986), carbon dioxide levels between 2,500 and 10,000 ppm increased the pH of raw breast muscles. The increased carbon dioxide exposure caused the utilization of stored glycogen in the bird in order to adapt to the stress, which in turn after death results in less glycogen for postmortem glycolysis and less lactic acid being produced resulting in a higher pH and darker colored meat.

Incomplete combustion of fuel during improper operation of heaters is responsible for the production of carbon monoxide in poultry houses (Sackett et al., 1986). Birds can also be exposed to carbon monoxide from the exhaust of trucks used in transportation from farm to the plant (Froning et al., 1969a). The a* (redness) values increased in raw poultry meat from birds exposed to carbon monoxide prior to slaughter. As the concentration of carbon monoxide is increased, the redness of the meat increased. Carbon monoxide will react and complex with
myoglobin and hemoglobin to form carboxymyoglobin and carboxyhemoglobin, respectively. A bright cherry red color is a characteristic of these pigments and will likely impart a red color into the meat tissues. Once carboxymyoglobin and carboxyhemoglobin pigments are formed in poultry meat, the pigments will likely remain stable throughout the cooking procedure, and influence the detrimental pink color defect occurring in uncured fully cooked poultry products (Froning et al., 1969a). However, in a study by Sackett et al. (1986), the carboxymyoglobin and carboxyhemoglobin pigments were formed upon exposure to carbon monoxide, but did not remain in the cooked product. The color of fully cooked poultry products were affected by carbon monoxide exposure causing a pink color to be formed in the product in research completed by Froning et al. (1969a). In birds exposed to carbon monoxide at the level of 1,000 ppm, the pH was increased in raw muscle when compared to lower levels of carbon monoxide exposure. The birds exposed to levels of carbon monoxide up to 750 ppm appeared to be excited and stressed which caused a lower pH in the muscle. The higher level of 1,000 ppm caused birds to become somewhat anesthetized and more tranquil prior to slaughter and is the reason for a higher pH than the stressed excited birds (Sackett et al., 1986). The exposure of birds to carbon monoxide can be a cause of the sporadic occurrence of the pink color defect. The environmental condition present inside poultry houses and on trucks during transportation should be monitored and controlled. The proper ventilation of poultry houses is needed to produce a quality bird, which will in turn produce a quality product.

The quality of meat produced from animals can be influenced by their feeding or feed withdrawal times. In research completed by Ngoka et al. (1982), a significant decline in final pH was found in poultry that was not removed from feed prior to slaughter as compared to birds properly removed from feed. The same results were discovered for pigs. The rapid decline in
final pH of animals not removed from feed prior to slaughter is a result of the rapid onset of rigor due to depletion of glycogen. The meat produced from animals not properly removed from feed experienced low muscle pH, low moisture content, and poor water holding capacity. Therefore, poor quality meat can be produced if the animal is not properly removed from feed prior to slaughter.

Another condition that has an effect on meat quality and color is stunning. Stunning is used to render the animal unconscious prior to slaughter to reduce the amount of struggle and stress. A struggling animal will produce low quality meat due to bruises on the meat as well as a low final pH due to an increase in the rate of postmortem glycolysis. The stunning techniques used are mainly used to reduce the amount of stress on the animal prior to slaughter, so higher quality meat will be produced. In research done by Thomson et al. (1986), the pH values of muscles from stunned birds was considerably higher than muscles from unstunned chickens immediately postmortem. It has been suggested that ante-mortem aerobic oxidation of glycogen is accelerated by the stunning process (Lee et al., 1976). This in turn causes less glycogen to be available for postmortem anaerobic glycolysis and consequently diminishes the production of lactate and pH decline. In research done by Young et al. (1996b), a higher ultimate meat pH was reached when increasing ante-mortem stunning time because the postmortem muscle glycogen levels were reduced by the increase in stunning time. The results showed that for each second increase in stunning time the pH is increased by 0.016 units. In this same experiment, it was determined that the color of the meat was not affected by stunning time. However, the shear value of the meat was increased when birds were stunned for more that four seconds, which means low quality tough meat was produced. Birds stunned from six to ten seconds were evaluated by a consumer panel as slightly tough in an experiment done by Lyon and Lyon.
In experiments done by Craig et al. (1999), birds were exposed to a high current stun, low voltage stun, or unstunned prior to slaughter. The birds that experienced the high current stun had a significantly higher raw muscle pH (6.41) than the low voltage stun raw muscle pH (6.17) or the unstunned group raw muscle pH (6.10). The high current stun and low voltage stun produced a higher redness (a*) value than the unstunned birds. The application of stunning could be a cause of the appearance of the pink color defect being currently found in fully cooked poultry meat. In contrast to the previous research, Ngoka et al. (1982) suggested that birds allowed to freely struggle during slaughter might produce an abnormal red coloration in the muscle due to high myoglobin concentration within the muscle. These findings can be traced back to the application of stress and excitation to the animal to produce a darker, redder muscle. This could also be a reason for the formation of pink color within fully cooked poultry meat.

Myoglobin is the principal heme pigment influencing meat color; along with cytochrome c and hemoglobin, which also contribute to the overall color of meat products (Ngoka and Froning, 1982). These heme pigments and their reactions with other constituents in the muscle have received blame for the occurrence of the pink color defect in fully cooked poultry meat by several researchers. The main pigment of concern to meat scientist and processors is myoglobin. Myoglobin is a monomeric, globular protein consisting of a heme group surrounded by a globin moiety and has a molecular weight of approximately 18,000 (Livingston and Brown, 1981; Seideman et al., 1984). The heme portion of myoglobin contains a centrally located iron (Fe) atom within four pyrrollic rings (Seideman et al., 1984). The valence state of the iron (Fe) atom and the ligand binding at the free bind site of the heme is of prime importance in determining the color of meat (Livingston and Brown, 1981; Seideman et al., 1984). The heme group of the myoglobin molecule is surrounded and protected by the globin moiety, which is a long chain
The concentration, oxidative state, and reactivity of myoglobin are factors that may affect meat color.

The key to understanding the color changes and reactions of myoglobin is the structure and chemistry of the iron atom located in the center of the heme moiety. The iron atom has eight valence electrons and is a third row transition metal, which means it contains unfilled energy levels below its valence electron levels. The absorption of visible light is attributed to the transition of electrons from filled orbitals to empty “3d” orbitals, which provides the color of myoglobin. Myoglobin in the \textit{in situ} or native state contains an iron atom that has lost two electrons due to its low electronegativity. The iron is in the ferrous form (Fe $^{++}$), but due to denaturation a third electron may be lost. This will change the iron to the ferric form (Fe $^{+++}$), which is unstable and will bind with other ligands to increase stability (Livingston and Brown, 1981).

The structure of myoglobin consists of eight $\alpha$-helical segments. The $\alpha$-helical segments are folded to give a flattened, globular shape, which provides a cleft to protect the heme moiety (Livingston and Brown, 1981). The heme moiety is held in position within the cleft by 25 amino acid residues, which makes the heme difficult to penetrate. The crack in the cleft that allows molecules, such as oxygen, to slide into the heme ring is small, thus protecting the heme from solvents and preventing the heme from binding with larger native muscle ligands. The cleft provides protection for the heme until the myoglobin molecule is denatured, which will result in an unfolding of the protein and exposure of the heme moiety (Price and Schweigert, 1987).

The myoglobin molecule usually has six ligands bound to the iron cation. The heme pyrrole nitrogens occupy four of the six coordination positions. The fifth coordination position (also called the proximal or axial position) in native myoglobin is occupied by histidine. The
sixth coordination position is usually bound to oxygen, but the oxygen can be removed and replaced with other ligands small enough to enter the protective cleft unless the protein has been denatured. The only ligands that can enter the undenatured myoglobin cleft and bind to the sixth coordination position are oxygen (O₂), carbon monoxide (CO₂), and nitric oxide (NO). However, when myoglobin is in the deoxymyoglobin or reduced myoglobin form, the sixth coordination position is left open (Livingston and Brown, 1981).

Once the protein has been unfolded due to denaturation, the cleft that protected the heme is diminished exposing the heme moiety. The exposure of the heme moiety allows ligands to complex the iron center to form ferrohemochromes or ferrihemochromes, depending upon the state of the iron. Ferrohemochromes are complexes formed by ferrous (Fe ++) myoglobin with a non-oxygen sixth ligand. Ferrihemochromes are complexes formed by ferric (Fe ++++) myoglobin with a non-oxygen sixth ligand. Several types of amino acid side chains can bind to the sixth coordination position of the heme moiety once the myoglobin molecule has been denatured. Different hemochromes are constantly interconverting because the exposed heme moiety can rapidly exchange ligands. The ferrohemochrome and ferrihemochrome complexes have a red pigmentation and have a visible spectrum similar to that of oxymyoglobin. However, the stability of the pigment is determined by rate of dissociation of the ligand bound to the sixth coordination position of the heme moiety. A ligand that dissociates quickly will oxidize causing color fading or browning to occur (Livingston and Brown, 1981). Certain amino acids, denatured proteins, and nitrogen containing ligands can bind to the heme moiety once myoglobin has been denatured to produce a double band spectrum and often a pink color (Cornforth et al., 1986).
The meat pH plays a major role in determining the color of a meat product due to the amount of amino acids or proteins that are available for heme complex formation. As the pH of a meat system increases, the reactivity or the concentration of reactable amino acid side chains will increase due to the increase in electronegativity of the amino acid side chains from globin or other proteins. Also, at a higher pH the hydrogen ion dissociation from the amino acid side chains will increase (Ahn and Maurer, 1990b). The higher pH allows more available amino acids or proteins to be available for hemochrome formation, thus affecting the color of the meat. In studies done by Trout (1989), high pH muscle (pH > 6.0) from beef, pork, and turkey was redder in appearance than low pH muscle (pH = 5.5) and appeared undercooked. The suggested explanation for the pink color that formed was the amount of myoglobin denatured was reduced by the high pH. In this same study, the myoglobin in turkey breast exhibited minimum denaturation at pH levels between 6.50 and 7.00, except when cooking temperatures were high enough to completely denature myoglobin. The average pH of most meat is 5.4 – 5.8, but in meat from stress prone animals it is not uncommon for the pH to be as high as 6.50 or greater.

The oxidation reduction potential (ORP) plays a huge role in determining the color of a meat product. ORP is the extent to which a chemical ion exchanges electrons, which leads to electrical charges, during a chemical reaction. It is a non-specific measure that is similar to the measurement of pH. The ORP is an important factor in pink color formation because it determines the state of the hemochrome (Fe + +)/hemichrome (Fe + + +) iron of globin hemochromes. The ORP also controls the ability of pigments to combine with ligands. The ORP is determined by the presence of reductants and is also a function of the pH of a meat product (Holownia et al., 2003). Cornforth et al. (1986), discovered that the presence of high reducing conditions allowed greater interconversion and reactivity of pigments that are
undesirable from the point of meat color. Under reducing conditions, the pink color intensity of the heme complexes formed from myoglobin and hemoglobin with naturally present ligands increased immensely (Ahn and Maurer, 1990a). As the pH of a meat product increases, the ORP tends to become more negative (reducing conditions) (Holownia et al., 2003). There are a number of reductants that can be used in meat to reduce heme pigments, some of which are endogenous to muscle tissue. The presence of intracellular nicotinamide coenzymes is related to meat reducing activities, such as nicotinamide adenine dinucleotide phosphate (NADP+, reduced form NADPH) and nicotinamide adenine dinucleotide (NAD+, reduced form NADH) (Giddings 1974, 1977; Livingston and Brown 1981; Renerre 1990). In a study conducted by Ahn and Maurer (1989b), cooked ground turkey meat exhibited the normal chromatic absorption peaks of oxymyoglobin (542 nm and 580 nm) when the ORP was in the -15 mV to -100 mV range. However, when the redox potential was reduced to -550 mV the myoglobin was fully reduced to the ferrous state and only one chromatic absorption peak was observed at 556 nm. In a similar study conducted by Cornforth et al. (1986), the heme iron was found to be in the ferrous state when the ORP was -550 mV or less, resulting in formation of only pink reduced hemochromes. Ahn and Maurer (1989b) found that the addition of processing ingredients, such as salt and phosphate, will cause a change in the ORP of a meat product. The native redox potential of a meat product is in the range of +90 mV to -50 mV and changes in the ORP could have a strong effect on pinking in cooked turkey breast meat. Cornforth et al. (1986) offered a suggestion to prevent the pink color defect in cooked meat under commercial conditions from occurring, it is necessary to maintain mildly oxidizing conditions.

The pink color defect that has plagued the poultry industry can be caused by the use of non meat ingredient in further processed products. The market for poultry products has shifted
over the years to be predominately further processed products. The consumers in the marketplace are shopping for convenience items and the further processed poultry products being produced are meeting these demands. However, to meet the demand created by consumers, processors have to continuously create new products, which involve the use of non meat ingredients. These ingredients being used help to improve the functionality of the product by increasing marination pickups and providing a wide variety of different flavors.

The main two ingredients used by processors to increase marination pickups are salt (NaCl) and phosphates. These two ingredients enter the meat tissue and bind with the actin and myosin proteins within muscle, which creates binding sites for water. The water molecules will bind with the salt and phosphate to become bound within the muscle, which will increase the marination pickup as well as, from a consumer aspect, increase the juiciness and flavor of the product.

However, a well known oxidizer of myoglobin is salt. There are two mechanisms by which salt promotes the oxidation of myoglobin. Lowering the buffering capacity of meat is the first mechanism. The dissociation of oxygen from the heme moiety, which oxidizes myoglobin to metmyoglobin, is a result of denaturation caused by low pH. Acids are oxidizing agents that will cause oxidation of myoglobin to occur, especially if the buffering capacity of the meat has been reduced by the addition of salt. A decrease in the uptake of oxygen is the second mechanism by which salt promotes the oxidation of myoglobin. The decrease in uptake of oxygen will cause low oxygen tensions within the meat resulting in oxidation of myoglobin (Seideman et al., 1984).

The use of additives such as salt and sodium tripolyphosphate (Na$_5$P$_3$O$_10$) may cause color problems in turkey breast because these additives alter the denaturation behavior of
proteins such as myoglobin (Trout, 1989). In research done by Trout (1989), he discovered that the percentage of myoglobin denatured increased linearly with increasing sodium chloride concentrations. Trout also concluded that salt will decrease the pinkness of cooked meat products. In the same experiment, Trout found that sodium tripolyphosphate increased the amount of denatured myoglobin, but it increased the pinkness of cooked meat products. Trout suggested that the reason for the pink defect was the increase in pH produced by the sodium tripolyphosphate. However, Trout stated that the tripolyphosphate ion reduces the pinkness of cooked meat products and recommended a neutral blend of phosphates, such as a mixture of sodium tripolyphosphate and sodium acid pyrophosphate, be used to inhibit the increase in pH caused by phosphates. In research completed by Ahn and Maurer (1990b), added salt solubilized myofibrillar proteins and increased the number of exposed reactable side chains, which increased the chance for heme complex formation. Also, this increases the chance for occurrence of the pink color defect, which may depend upon which heme complex was formed from the solubilized proteins and exposed side chains.

Increases in water holding capacity and protein solubility make salt a commonly used additive in heat treated meat products. Salt accomplishes this task by increasing the ionic strength of the meat product, which allows more proteins to be solubilized. The ionic strength of a meat product is the amount of dissociated ions available in a meat product. The temperature at which denaturation of myoglobin occurs is influenced by the ionic strength of a meat product. In research done by Kristensen and Purslow (2001) showed that the heme molecule was protected from heat induced destruction by the addition of salt. The meat products produced using salt had greater amounts of heme iron than meat products produced without salt. Also, the meat products produced with added salt showed decreases in cooking loss as compared to the
meat products produced without salt addition. The authors suggested that the increase in ionic strength provided the protecting effect on the heme molecule by influencing the rate of chemical reactions.

The ionic strength of a meat product is continuously changing from the time the animal is harvested to the table of a consumer. In studies done by Feidt and Brun-Bellut (1999), the ionic strength of a meat product begins to increase during the development of rigor mortis due to ion release. Ions are released by lactate and organic acids during anaerobic glycolysis. The ionic strength of a meat product was not affected by chilling treatments. The authors concluded that ionic strength plays a major a function in post mortem muscle changes.

The pH of a meat product can influence the ionic strength. As the pH of a meat product decreases, the amount of available ions increases (Feidt and Brun-Bellut, 1999). In an experiment by Gil et al. (1998), the authors discovered that protein solubility of different types of meat, including poultry, increased in accordance with the increasing ionic strength. The solubility of the myofibrillar protein extracts accounted for most of the difference found. The characteristics of the muscle and the myofibrillar protein solubility are modified by changes in the salt concentration or in the pH of meat (Dilber-Van Griethuysen and Knight, 1991). However, the protein extractability is highly dependent upon the ionic strength and pH of the salt solution used on the meat product (Franks 1993).

The water holding capacity of muscle and muscle products are known to be influenced by salts and pH. Improvement in physical properties of products and increased profits are a result of increased water uptake and water holding capacity of muscle protein gels; therefore, this becomes an important issue for processors. The results from studies done by Kristinsson and Hultin (2003) have shown that increases in pH cause myofibrils to expand. The expansion of the
myofibrils will allow more water to be taken up and less to be lost. It is common belief that only under high salt conditions that proper gelation can be achieved because of myofibrillar protein solubility, but Kristinsson and Hultin (2003), have shown that strong gels with excellent water holding capacity can be formed under low ionic strength conditions. The myofibrillar proteins were essentially insoluble under these low salt conditions when the gels were formed. The authors also demonstrated that the gels formed at pH 7.0 with low ionic strength had greater gelation and water holding capacity than the gels form at lower pH values. The authors stated that less water was taken up by chicken breast when the ionic strength was increased because the addition of salt caused a decrease in filament spacing and that the electrostatic repulsion within the cell matrix was lowered. The authors suggested the theory that the driving force for water uptake is repulsion created by the negative charges of the proteins. In research completed by Feng and Hultin (2001), chicken breast muscle proteins formed stronger, more evenly distributed gels at pH 7.0 with low ionic strength than at pH 6.4 with low ionic strength.

The use of phosphates as an additive in meat products is a common practice among processors to increase water holding capacity and tenderness of the products. There are many different types of phosphates being used in meat products, which include the most commonly used sodium tripolyphosphate along with sodium acid pyrophosphate and sodium orthophosphate. There has been an increase in the use of inorganic, alkaline sodium phosphates in meat products of beef, chicken, and pork due to their beneficial effects. The use of sodium phosphates improves functionality, palatability, and storage stability of meat products. Sausages and restructured meat product processors are using polyphosphates to enhance water holding capacity, color, flavor, and texture, which in turn improves quality of the products (Lee et al., 1998). The results of a study done by McGee et al. (2003) indicated that the use of sodium
tripolyphosphate increased tenderness and sensory characteristics as well as decreased cooking loss and lipid oxidation.

During the storage of meat and precooked meat products, lipid oxidation can cause rancidity and off flavor problems. Phosphates are used to bind prooxidants in a meat product and reduce off flavor as determined by a trained sensory panel. Also, the pH and ionic strength of meat products are increased with the addition of phosphates (Cheng and Ockerman, 2003). The increase in pH of a meat product by phosphates improves water holding capacity (Allen et al., 1998). Phosphates improve the functional properties of PSE meat by increasing the pH and ionic strength. In research done by Torley et al. (2000), cook loss of PSE meat was reduced by polyphosphate when compared to PSE meat without polyphosphate addition. The same results were found when normal muscle was used in the place of PSE muscle. However, during this experiment, less protein was extracted from the PSE muscle when compared to the normal muscle when a combination of pyrophosphate and salt was added. Also, a large percentage of the PSE muscle myofibrils did not swell, whereas, all of the normal muscle myofibrils swelled equally. The myofibrils of the PSE muscle did not swell equally because some of the myosin heads were partially denatured, which prevents the actomyosin from being split by the polyphosphate and limits swelling. The authors also discovered that the use of polyphosphates in a high ionic strength solution will cause the non-denatured PSE muscle myofibrils to swell and produce a three dimensional protein network that will bind water and retain it after heating. However, the addition of polyphosphates will only improve the functionality of non-denatured PSE myofibrillar, but will not improve the denatured muscle proteins.

The use of added phosphates can produce color problems in products, which can be linked to the occurrence of the pink color defect in uncured, fully cooked poultry products. In
research done by Young and Lyon (1994), turkey breast muscle color was altered due to the increase in pH caused by the addition of sodium tripolyphosphate prior to the resolution of rigor. In this experiment, the CIE a* value or redness of the turkey breast meat was increased by the addition of sodium tripolyphosphate. The authors suggest that the increased pH inhibited myoglobin denaturation. The sensitivity of myoglobin to heat denaturation was shown in model systems to decrease as muscle pH increased (Trout 1989). However, in studies done by Young et al. (1996a), the addition of sodium tripolyphosphate to postrigor muscle decrease the CIE a* values in cooked turkey breast meat. In a comparison of the studies completed by Young and Lyon (1994) and Young et al. (1996a), the addition of phosphates to prerigor muscle increased the pink color of the uncured, fully cooked turkey breast meat; whereas, addition of phosphates postrigor decreased the pinkness of the uncured, fully cooked turkey breast meat. The reason being the myoglobin in the postrigor muscle was already denatured due to the decrease in pH during rigor mortis. The addition of phosphate to prerigor muscle kept the pH high providing a protective effect for myoglobin, which produced a product with the pink color defect due to undenatured myoglobin.

Another benefit of phosphate addition comes from a microbial aspect. In a study done by Pathgeber and Waldroup (1995), the microbial population of poultry meat was altered by chilling the chicken carcasses in commercial blends of polyphosphates. The use of trisodium phosphate (TSP) is an effective antimicrobial agent in controlling *Campylobacter*, *E. coli*, *L. monocytogenes*, *Salmonella*, *S. aureus*, and psychrotrophs. Many hypotheses have been made to explain the antibacterial mechanism through which TSP reduces microorganisms, but it is not fully understood. Some researchers have speculated that a thin layer of fat and bacteria are removed from the surface of poultry skin by TSP. Others have suggested that TSP chelates
essential metal ions in the cell walls, which reduces the number of microorganisms (Vareltzis et al., 1997). In a study completed by Vareltzis et al. (1997), the use of sodium tripolyphosphate reduced the amount of spoilage bacteria on poultry carcasses. The carcasses treated with sodium tripolyphosphate exhibited less slime and off odors when compared to controls not treated with sodium tripolyphosphate. Also, the treated carcasses had three extra days of shelf life when stored at 4°C when compared to the untreated carcasses.

Another non meat ingredient commonly used in meat products that has similar microbial reduction capabilities is sodium citrate. Sodium citrate uses a combined effect of cation chelation and mineral deprivation of the bacteria to provide microbial reduction (Miller et al., 1993). In studies done by Long and Phillips (2003), the authors concluded that sodium citrate provided a multiple hurdle approach at controlling populations of spoilage and pathogenic bacteria on, or in, meat or meat products.

In meat products, sodium citrate is commonly used in combination with phosphate and in some cases takes the place of phosphate in phosphate free meat products. Sodium citrate is used to improve the texture and water holding capacity of meat products by raising the pH (Ruusunen et al., 2003). Sodium citrate has recently received attention by researchers as a possible inhibitor of the pink color defect being found in uncured, fully cooked poultry products because it is a chelator that has the potential to bind the heme iron of myoglobin. This prevents other ligands from binding to the heme that could possibly cause formation of the pink color defect (Sammel and Claus, 2003). In a study done by Sammel and Claus (2003), sodium citrate did not have any affect on the pinkness of intact turkey breast that were treated with sodium nitrite and nicotinamide, which are pink inducing agents. However, sodium citrate effectively reduced pinking in ground turkey meat that was treated with both pink inducing agents, especially
nicotinamide. The authors suggested that the pinkness of the intact turkey breast was not reduced by sodium citrate because it was unable to access the heme of myoglobin and prevent the pink color generating ligands from binding.

Citric acid is also a chelator that has similar effects as sodium citrate. In an experiment conducted by Kieffer et al. (2000), citric acid inhibited the pink color defect from forming in ground turkey meat. In a study done by Sammel and Claus (2003), citric acid used at 0.2% and 0.3% (meat weight basis) effectively reduced pinking in ground turkey meat treated with known pink inducing agents, sodium nitrite and nicotinamide. In the same study, citric acid used at 0.1%, 0.2%, 0.3%, and 1.0% (meat weight basis) had no effect on pinking of intact turkey breast treated with sodium nitrite and nicotinamide. The authors suggested that the pinkness of the intact turkey breast was not reduced by citric acid because it was unable to access the heme of myoglobin and prevent the pink color generating ligands from binding. In another study researched by Yang and Chen (1993), chicken breast marinated with citric acid had reportedly decreased external and internal CIE a* values (redness). The disagreement between the previous two studies can possibly be contributed to the use of turkey rather than chicken.

The purpose of the research conducted is to explore ways to reduce the occurrence of the pink color defect in uncured, fully cooked chicken breast by use of marinade ingredients and ionic strength. The pink color defect has had a devastating effect on the poultry industry by reducing consumer satisfaction and profits. In response to this devastating effect the use of a sodium citrate and citric acid combination along with a blend of sodium phosphates was used in different marinade formulation with varying ionic strengths on chicken breast muscle to attempt to reduce the formation of pink color on the interior of the breast muscle.
3.3 Materials and Methods

Chicken Breast Samples

Boneless, skinless chicken breasts (Pectoralis major) were obtained from Wayne Farms located in Pendergrass, Ga. The chicken breasts were selected and visually sorted from the debone line based on the three color groups: lighter than normal ("light"), normal ("normal"), and darker than normal ("dark") (Fletcher, 1999; Fletcher et al., 2000). The color measurements were taken once the breasts were visually sorted from the line using a Minolta colorimeter (model # CR-200, Japan) on the medial surface (bone side) of the breasts. The chicken breasts were sorted into two groups, normal color muscle with a CIE L* (Lightness) value range of 47 < CIE L* < 50 and dark color muscle with a CIE L* value range of CIE L* < 47. The light color breasts that had a CIE L* > 50 were returned back to the debone line. The color measurements were taken to ensure the breasts were sorted into each group correctly based on the color ranges set for this experiment. The breasts were then packaged into plastic bags keeping the normal and dark groups separate and placed into an ice filled cooler to be returned back to the lab for experimentation.

The boneless chicken breasts were collected in ten pound increments for each color group. There was a total of sixteen experiments ran using one of four different marinades. The normal color chicken breasts were exposed to each of the four marinades of varying pH and ionic strength (Tables 3.1-3.4) with each experiment ran in duplication. The dark color chicken breasts were exposed to each of the four marinades just as the normal color group with each experiment ran in duplication. A total of eight trips were made to the processing plant to retrieve ten pounds of normal color breasts and ten pounds of dark color breasts on each trip.
Once arriving back to the University of Georgia, the chicken breasts were removed from the ice filled cooler and placed into walk-in coolers located in the pilot plant at the Food Science and Technology Department building. The walk-in coolers are held at a constant temperature of 4°C. The chicken breasts were held in the walk-in coolers for approximately 24 hours postmortem based on the approximate time of slaughter.

**Sample Preparation**

Each experiment ran used a ten pound batch of either normal color or dark color boneless, skinless chicken breasts. After the 24 hour postmortem time period, the chicken breast were removed from the walk-in cooler and tagged with yellow or blue numbered tags for the normal color or dark color groups, respectively, so the individual breast could be tracked throughout the experiment from the raw state of the muscle through the final cooking procedure. Once the breasts were tagged, each breast was weighed to obtain the raw weight of each breast. The batch size for each experiment was approximately ten pounds (raw weight) or 22-23 breasts.

**pH, ORP, and Color Measurements**

The pH of each individual breast was taken at the beginning of each experiment at the raw state as well as after the marination and cooking procedures. The pH measurement was taken at the top, thick portion of the breast using a ThermoOrion pH/ISE meter (model # 710A+, Beverly, MA) attached to an Orion spear tipped pH probe (model # KnipHE 7120BN, United Kingdom). The pH meter was calibrated with two calibration points with the pH values of 6.997 and 4.010 before taking any pH measurements at each of the raw, marinated, and cooked steps.

The oxidation reduction potential (ORP) of each individual breast was taken at the beginning of each experiment at the raw state as well as after the marination and cooking procedures. The ORP measurement was taken on the medial surface (bone side) of each breast.
A sharp knife was used to slice back a small, thin portion of the meat to expose a fresh surface for the ORP measurement to be taken. The ORP was taken by using a ThermoOrion pH/ISE meter (model # 710A+, Beverly, MA) attached to a Cole Parmer oxidation reduction potential surface probe (model # u-27003-40, Vernon Hills, IL). The first ORP measurement taken at the beginning of each step was taken after three minutes to allow the ORP meter to stabilize. Subsequent ORP measurements were taken after one minute because the ORP meter was previously stabilized.

The color measurements throughout the experiment were taken with a Minolta Colorimeter (model # CR-200, Japan) that was calibrated using a white calibration tile before each of the raw, marination, and cooking steps. In the raw and marinated states of the breasts, the CIE L* color measurement was taken on exterior, upper thick portion of the breast on the medial surface. Once the breasts were cooked, the upper, thick portion of the breast was sliced open at a 45° angle and the CIE L*, a*, and b* values were immediately taken to obtain an internal color measurement using the Minolta Colorimeter.

**Marinades**

In this experiment, four different marinades treatments were used with varying pH values and ionic strengths (Tables 3.1-3.4). The normal color and dark colored groups of chicken breast were marinated using each of the four marinades with each marination completed in duplication. Each of the four different marinades was formulated in 1000 milliliter batches. The ingredients were carefully measured out using a Mettler Toledo analytical balance (model # AB104-S, Switzerland). The marinade was warmed and stirred using a heat/stir plate to ensure each ingredient was completely dissolved and distributed throughout the aqueous solution. The four different marinade treatments used in this experiment were:
• Treatment 1: pH = 5.7 with an ionic strength (u) = 0.17
• Treatment 2: pH = 6.0 with an ionic strength (u) = 0.21
• Treatment 3: pH = 5.7 with an ionic strength (u) = 0.22
• Treatment 4: pH = 6.0 with an ionic strength (u) = 0.25

The formulations for each marinade are listed in Tables 3.1-3.4. A sample ionic strength calculation for Treatment 1 is presented in Appendix 3.1.

**Marination Procedure**

The chicken breasts were marinated using a Wolftech, Inc. vacuum cold massager (model # 1120, Kingston, NY). The marinade was added at a 15% pump based on the raw weight of the batch, which was approximately 10 pounds or 22 - 23 breasts. The breasts were subjected to a 27in Hg vacuum and tumbled for 20 minutes at 20 revolutions per minute. The marination procedure was conducted in a walk-in cooler, where the temperature of the marinade, tumbler, and chicken was held constant at 4°C. Upon completion of the tumble, the breasts are allowed to rest inside the tumbler for 10 minutes. The breasts were then removed from the tumbler to allow each breast to be weighed. The percentage of marinade pickup was calculated using the marinated weight and the previously recorded raw weight by using the formula

\[
\% \text{ marinade pick up} = \frac{\text{Marinated weight} - \text{Raw weight}}{\text{Raw weight}} \times 100
\]

**Cooking Procedure**

The cooking procedure used for this experiment was held constant and the program used is listed in Table 3.5. The marinated chicken breasts were placed on wire racks located on a roll in truck of the Alkar processing oven (model # 1000, Lodi, WI). Once the chicken breasts were in place, temperature probes were inserted into five randomly selected chicken breasts throughout the processing oven to get evenly distributed temperature measurements because one
certain point might not attain the same temperature as another due to cold spots within the oven. The temperature was measured to ensure a proper internal cooking temperature of 166°F was reached. Upon completion of the cooking procedure, the chicken breasts were allowed to cool to approximately room temperature before final measurements were taken. The weight of each cooked breast was measured to allow calculation of the cook and process yield for each run of the experiment by using the following formulas:

- **Cook Yield** = \( \frac{\text{Cook weight}}{\text{Marinated weight}} \) x 100
- **Process Yield** = \( \frac{\text{Cook weight}}{\text{Raw weight}} \) x 100

**Statistical Analysis**

The statistical analysis for the data complied during this research was accomplished using the analysis of variance option of the general linear models (GLM) procedure of the Statistical Analysis Systems (SAS) program. The means for each of the four treatments within the normal color breast and dark color breast groups were tested for significance using Least Squares Means test with an adjustment for multiple comparisons. A logistic regression model procedure of the SAS program was used to develop the pinking probability equation. The results measured and tested for this experiment were pH, ORP, percent pink, yield, and color. The averages, standard deviations, and significant differences for measurements are illustrated in Table 3.6a, 3.6b.
Table 3.1: Marinade formulation for Treatment I  
(pH = 5.7 with an ionic strength of 0.17)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% in meat(^1)</th>
<th>% pump(^2)</th>
<th>Grams in Marinade(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Tripolyphosphate</td>
<td>0.22</td>
<td>15.0</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Tetrasodium Pyrophosphate</td>
<td>0.08</td>
<td>15.0</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>0.50</td>
<td>15.0</td>
<td>33.33 g</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>0.10</td>
<td>15.0</td>
<td>7.0 g</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
<td>15.0</td>
<td>23.33 g</td>
</tr>
<tr>
<td>Water</td>
<td>98.75</td>
<td>15.0</td>
<td>916.34 g</td>
</tr>
</tbody>
</table>

1. % in meat = projected percent of ingredient within the chicken breast muscle after vacuum tumble marination at a 15% pump
2. % pump = percent of marinade added to each batch of chicken breast for vacuum tumble marination (meat weight basis)
3. Grams in Marinade = actual weight in grams of each ingredient added to Treatment I marinade
Table 3.2: Marinade formulation for Treatment II  
(pH = 6.0 with an ionic strength of 0.21)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% in meat$^1$</th>
<th>% pump$^2$</th>
<th>Grams in Marinade$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Tripolyphosphate</td>
<td>0.10</td>
<td>15.0</td>
<td>7.0 g</td>
</tr>
<tr>
<td>Tetrasodium Pyrophosphate</td>
<td>0.03</td>
<td>15.0</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Orthophosphate</td>
<td>0.02</td>
<td>15.0</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium Acid Pyrophosphate</td>
<td>0.15</td>
<td>15.0</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Salt</td>
<td>0.86</td>
<td>15.0</td>
<td>57.33 g</td>
</tr>
<tr>
<td>Water</td>
<td>98.84</td>
<td>15.0</td>
<td>922.67 g</td>
</tr>
</tbody>
</table>

1. % in meat = projected percent of ingredient within the chicken breast muscle after vacuum tumble marination at a 15% pump  
2. % pump = percent of marinade added to each batch of chicken breast for vacuum tumble marination (meat weight basis)  
3. Grams in Marinade = actual weight in grams of each ingredient added to Treatment II marinade
Table 3.3: Marinade formulation for Treatment III  
(pH = 5.7 with an ionic strength of 0.22)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% in meat&lt;sup&gt;1&lt;/sup&gt;</th>
<th>% pump&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Grams in Marinade&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Tripolyphosphate</td>
<td>0.22</td>
<td>15.0</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Tetrasodium Pyrophosphate</td>
<td>0.08</td>
<td>15.0</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>0.50</td>
<td>15.0</td>
<td>33.33 g</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>0.10</td>
<td>15.0</td>
<td>7.0 g</td>
</tr>
<tr>
<td>Salt</td>
<td>0.58</td>
<td>15.0</td>
<td>38.67 g</td>
</tr>
<tr>
<td>Water</td>
<td>98.52</td>
<td>15.0</td>
<td>901.00 g</td>
</tr>
</tbody>
</table>

1. % in meat = projected percent of ingredient within the chicken breast muscle after vacuum tumble marination at a 15% pump
2. % pump = percent of marinade added to each batch of chicken breast for vacuum tumble marination (meat weight basis)
3. Grams in Marinade = actual weight in grams of each ingredient added to Treatment III marinade
Table 3.4: Marinade formulation for Treatment IV  
(pH = 6.0 with an ionic strength of 0.25)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% in meat(^1)</th>
<th>% pump(^2)</th>
<th>Grams in Marinade(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Tripolyphosphate</td>
<td>0.10</td>
<td>15.0</td>
<td>7.0 g</td>
</tr>
<tr>
<td>Tetrasodium Pyrophosphate</td>
<td>0.03</td>
<td>15.0</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Orthophosphate</td>
<td>0.02</td>
<td>15.0</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium Acid Pyrophosphate</td>
<td>0.15</td>
<td>15.0</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Salt</td>
<td>1.1</td>
<td>15.0</td>
<td>73.33 g</td>
</tr>
<tr>
<td>Water</td>
<td>98.60</td>
<td>15.0</td>
<td>906.67 g</td>
</tr>
</tbody>
</table>

1. % in meat = projected percent of ingredient within the chicken breast muscle after vacuum tumble marination at a 15% pump
2. % pump = percent of marinade added to each batch of chicken breast for vacuum tumble marination (meat weight basis)
3. Grams in Marinade = actual weight in grams of each ingredient added to Treatment IV marinade
Table 3.5: Cooking program used by the Alkar processing oven to cook the marinated boneless, skinless chicken breast for each batch used in this experiment.

<table>
<thead>
<tr>
<th>Step #</th>
<th>Step Time (min)</th>
<th>Dry Bulb Temp (° F)</th>
<th>Wet Bulb Temp (° F)</th>
<th>% Relative Humidity</th>
<th>Internal Temp (° F)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>00:20</td>
<td>140</td>
<td>108</td>
<td>35</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>00:15</td>
<td>150</td>
<td>123</td>
<td>45</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>00:20</td>
<td>170</td>
<td>147</td>
<td>55</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>00:10</td>
<td>180</td>
<td>165</td>
<td>70</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>00:01</td>
<td>190</td>
<td>177</td>
<td>75</td>
<td>166</td>
</tr>
</tbody>
</table>

1. Internal temperature = temperature measured in the center of chicken breast by temperature probes attached to the Alkar processing oven (temperature was measured on five randomly selected chicken breast within each batch to account for any cold spots within the processing oven).
3.4 Results and Discussion

Pinking

The number of chicken breasts with pinking or pinkness was measured in this study to determine the percentage of pinking for each of the four treatments within the normal color breast and dark color breast groups. The pinking threshold used was determined by Holowonia et al. (2002), which was set at the CIE $a^* \geq 3.8$. The pinking threshold determined by Holowinia et al. was a result of research that compared results from colorimetric data and visual examination that established the least visible pinking at CIE $a^* = 3.8$. Breast with CIE $a^* \geq 3.8$ were considered to have pinking or the pink color defect. This pinking threshold used was lower than those reported by other authors, which used $a^*$ values of greater than 4.0 for poultry white meat (Ahn and Maurer, 1989b; Heaton et al., 2000).

The normal color breast group contained less pinking for each of the four treatments used in this experiment when compared to the dark color breast group (Figure 3.1). This was expected because dark color breast are more susceptible to pinking due to higher muscle pH and high initial color values. The percent pinking for the normal color breast group ranged from 0% - 11.11%, with treatment I having the minimum amount and treatment III having the maximum (Figure 3.1). These two treatments both involved a marinade pH of 5.7 with varying ionic strength levels. Treatment I and Treatment III marinades contained a combination of 0.5% sodium citrate and 0.10% citric acid (meat weight basis (MWB)) with varying amount of sodium chloride (Tables 3.1-3.4). The amount of sodium chloride was increased from 0.35% MWB in Treatment I to 0.58% MWB in Treatment III to produce varying marinade ionic strengths of 0.17 and 0.22, respectively. However, Treatment III exhibited the most pinking of treatments among the normal color breast group and Treatment I was void of any pink color formation.
Treatment II and Treatment IV marinades from the normal color breast group involved a marinade pH of 6.0 and contained a combination of 0.01% MWB sodium orthophosphate and 0.15% MWB sodium acid pyrophosphate (Tables 3.1-3.4). The ionic strength of the two marinades was varied by increasing the sodium chloride concentration from 0.86% MWB in Treatment II to 1.1% MWB in Treatment IV to produce marinade ionic strengths of 0.21 and 0.25, respectively. The percentage of pinking increased along with the marinade ionic strength level producing 2.27% pinking in Treatment II and 8.89% pinking in Treatment IV (Figure 3.1).

The percentage of pinking was increased in the dark color breast group when compared to the normal color breast group and produced a percent pinking range of 10.87% - 28.26% (Figure 3.1). Treatment I produced the lowest amount of pinking for the dark color breast group, just as in the normal color breast group, but Treatment II produced the most pinking. Treatment I and III produced the lowest amounts of pinking for the dark color breast group with percentages of 10.87% and 17.78%, respectively. These two marinades were the same as used in the normal color breast group and contained a combination of sodium citrate and citric acid (Tables 3.1-3.4). The highest amounts of pinking for the dark color breast group was exhibited by Treatment II and Treatment IV with pinking percentages of 28.26% and 20.00%, respectively (Figure 3.1). These two marinades are the same as used in the normal color breast group and contain a combination of sodium orthophosphate and sodium acid pyrophosphate. The percent pinking seemed to follow the increasing ionic strength for Treatments I and III, but this was not the case between Treatments II and IV.

The amount of pink color formation among the four treatments was at a minimum in Treatment I for both the normal color breast and dark color breast groups. The amount of pinking that was present among the four treatments seemed to increase as the ionic strength of
the marinade increased except between Treatment II and Treatment IV of the dark color breast group. The ionic strengths of the marinades were increased by increasing the sodium chloride content. The increase in salt content may cause more proteins and protein side chains to be denatured and become available for binding with the sixth coordinate position of myoglobin to produce a pink color forming hemochrome. In research done by Trout (1989), the percentage of myoglobin denatured increased linearly with increasing sodium chloride concentrations. Trout also concluded that salt will decrease the pinkness of cooked meat products, which was not the case in our research. However, in research completed by Ahn and Maurer (1990b), added salt solubilized myofibrillar proteins and increased the number of exposed reactable side chains, which increased the chance for heme complex formation. Also, this increases the chance for occurrence of the pink color defect, which may depend upon which heme complex was formed from the solubilized proteins and exposed side chains. The results of pinking produced in the current experiment were in agreement with Ahn and Maurer, in which the increasing sodium chloride concentration produced a greater chance for pink color formation.

The treatments that contained the combination of sodium citrate and citric acid produced less pink color than the treatments that included sodium orthophosphate and sodium acid pyrophosphate. Sodium citrate has recently received attention as a possible inhibitor of the pink color defect being found in uncured, fully cooked poultry products because it is a chelator that has the potential to bind the heme iron of myoglobin. This prevents other ligands from binding to the heme that could possibly cause formation of the pink color defect (Sammel and Claus, 2003). In a study by Sammel and Claus (2003), sodium citrate did not have an effect on the pinkness of intact turkey breast that were treated with sodium nitrite and nicotinamide, which are pink inducing agents. However, sodium citrate effectively reduced pinking in ground turkey
meat that was treated with both pink inducing agents. Sammel and Claus suggested the pinkness of the intact turkey breast was not reduced by sodium citrate because it was unable to access the heme of myoglobin and prevent the pink color generating ligands from binding.

Citric acid is also a chelator that has similar effects as sodium citrate. In an experiment conducted by Kieffer et al. (2000), citric acid inhibited the pink color defect from forming in ground turkey meat. Sammel and Claus (2003) found citric acid to have no effect on pinking of intact turkey breast. The pinkness of the intact turkey breast was not reduced by citric acid in the same manner as sodium citrate because it was unable to access the heme of myoglobin and prevent the pink color generating ligands from binding. In another study researched by Yang and Chen (1993), chicken breast marinated with citric acid decreased external and internal CIE a* values (redness). The disagreement between the previous two studies may possibly be contributed to the use of turkey rather than chicken.

However, in the current research the sodium citrate was combined with citric acid and a sodium tripolyphosphate/tetrasodium pyrophosphate blend as well as salt to form a marinade, which was vacuum tumbled to ensure access into the muscle. The marinade was added in 15% increments that were consistent between treatments I and III for normal and dark color breast groups. The percentage of marinade picked up by the breast was not statistically different (P > 0.05) for any of the four treatments for the normal color breast group or for the dark color breast group (Figure 3.2). The reduction in pinking for Treatment I for the normal and dark color is possibly due to the use of sodium citrate and citric acid along with a decreased ionic strength.
**pH and ORP Conditions**

The raw meat pH, marinated meat pH, and cooked meat pH for the two color groups were different with the dark color group having consistently higher pH values than the normal color group (Figure 3.3). The average raw pH values among the four treatments ranged from 6.01 - 6.17 and 6.18 – 6.33 for the normal color and dark color breast group, respectively. The average marinated pH values among the four treatments ranged from 6.08 – 6.16 and 6.21 – 6.31 for the normal color and dark color breast group, respectively. The average cooked pH values among the four treatments ranged from 6.18 – 6.25 and 6.23 – 6.32 for the normal color and dark color breast group, respectively. The higher pH values in the dark color breast group was expected and agrees with results from a study conducted by Fletcher (1999), in which dark breast muscle had higher pH values than lighter colored breast muscle. Higher pH muscle appears darker and redder because it has a higher water binding capacity, which is associated with greater translucence and less light scattering to allow greater light penetration and absorption by myoglobin (MacDougall 1982; Bendall and Swatland, 1988).

The normal color breast group experienced less pink color formation than the dark color breast group (Figure 3.1). The normal color breast group may have exhibited a less number of breast pink because of a lower pH and more denatured myoglobin within the muscle. As the pH of a muscle rises above 6.0, the myoglobin becomes less susceptible to denaturation (Janky and Froning, 1972,1973). A model system developed by Trout (1989) was used to show the heat denaturation sensitivity of myoglobin decreases as muscle pH increases. Also, if the meat pH reaches levels of higher than 6.3, which was the case in the current study for the dark color breast group, the side chains of nicotinamide and meat proteins (histidine, cysteine, and methionine) start to form heme complexes and exhibit a pink coloration in meat (Ahn and Maurer, 1990b).
The iron of the heme moiety shifts predominantly to the ferrous (Fe ++) as the pH rises above 6.1, where as a lower pH accelerates the conversion of the ferrous state to the ferric (Fe ++++) state (Ahn and Maurer, 1990b). The pink color generating heme complexes are primarily formed from the ferrous state, so as the pH of a muscle increases the possibility of pink color formation increase due to more iron of the heme moiety being in the ferrous state. The structure of a high pH muscle is much tighter when compared to a lower pH muscle because the high pH will allow muscle fibers to bind more water and become swollen and tightly packed. The tighter muscle structure of a high pH muscle allows less oxygen penetration, thus lowering the oxidation reduction potential (ORP) and creating reducing conditions within the muscle (Hunt and Kropf, 1987).

The ORP in the current study was measured at the raw, marinated, and cooked phases of each breast (Figure 3.4). The raw ORP values ranged from -4.44 mV to -38.75 mV and -16.303 mV to -45.827 mV for normal and dark color breast groups, respectively. The marinated ORP values ranged from -24.72 mV to -37.73 mV and -36.77 mV to -50.03 mV for normal and dark color breast groups, respectively. The cooked ORP values ranged from -78.57 mV to -101.21 mV and -109.00 mV to -113.90 mV for normal and dark color breast groups, respectively.

The ORP is a function of the pH and tends to become more negative (increasing reducing conditions) at a high pH (Holownia et al., 2003; Antonini and Brunori, 1971). The state of the iron of globin hemochromes (hemochrome(Fe++)/hemichrome(Fe+++)) and the ability of pigments to combine with other molecules is largely determined by the ORP, which make it an important factor in pink color formation (Holownia et al., 2003). Under reducing conditions, the pink color intensity of the heme complexes formed from myoglobin and hemoglobin with ligands increases tremendously (Ahn and Maurer 1990a). The reactivity and interconversion of
pigments that is unfavorable from the point of meat color is increased under high reducing
conditions (Cornforth et al., 1986; Ahn and Maurer, 1989b, 1990b).

The color of meat is influenced by any condition, such as irradiation, that can change the
oxidative status of the heme iron and produce a ligand compound that can easily bind to the sixth
coordination position of the ferrous iron heme moiety (Nam and Ahn, 2002b). In a study
conducted by Du et al. (2002), the CIE a* value (redness) of irradiated meat was increased by
irradiation due to the production of CO and reduced oxidation reduction potential. When non
irradiated meat was exposed to the same concentration of CO, the color of the meat was not
affected. The reduced oxidation reduction potential allowed the CO to form complexes with the
heme pigments and/or become the sixth ligand of myoglobin to produce the pink color defect in
irradiated meat products.

In the current study, the dark color breast group expressed greater reducing conditions
than the normal color breast group (Figure 3.4), which may be the reason for a greater number of
breasts that exhibited the pink color defect. When the results for each treatment were broken
down within the normal color breast group, Treatment III had the most samples with the pink
color defect (11.11%) as well as the lowest ORP when compared to Treatment I, which had no
samples with the pink color defect (Figure 3.5). However, the marinated and cooked pH values
were similar between the two treatments, but the initial raw pH was higher for Treatment III
(Figure 3.3). The increase in the number of breast with pink color for normal color in Treatment
III may be contributed to the increased reducing condition in combination with a muscle pH
value greater than 6.2 (Figure 3.6). The increased ORP (less reducing condition) may possibly
be the reason why Treatment I of the normal color breast group was devoid of any samples
experiencing the pink color defect.
The treatments for the dark color breast group with the minimum (Treatment I) and the maximum (Treatment II) number of breast with the pink color defect had marinated ORP and cooked ORP values that were not statistically different (P > 0.05). However, Treatment I had a considerably higher raw ORP (less reducing conditions) than any of the other treatments and may be the reason for less pink sample. The pH values of the four treatments in the dark color breast group were around 6.3, which could cause more protein side chains and amino acids to be available for heme complex formation. The increased number of available ligands because of the high pH in combination with higher reducing condition may be the reason for more pink color formation.

**Pinking Prediction**

A logistic regression model was used to develop a pinking prediction equation. The equation was developed from the cooked pH, cooked ORP, and cooked CIE a* values of individual breasts rather than from mean values reported (Table 3.6a, 3.6b). The pinking threshold of CIE a* ≥ 3.8 was used to determine whether a sample was pink (Holownia et al., 2002). The individual breast cooked ORP and cooked pH measurements were used because the mean values did not express the distribution of the values. Frequency histograms (Figures 3.7a, 3.7b, 3.7c, 3.7d) were used to show differences in the distribution of individual breast cooked ORP and cooked PH measurements among different treatments, whereas the means among the different treatments were not statistically different. There is a bimodal pinking threshold value that exists between cooked pH and cooked ORP. The pinking prediction equation listed below uses a function of cooked pH and cooked ORP to predict the percentage of samples devoid of the pink color defect.
\[
P(\text{no pink}) = \frac{e^{(6.4154 - (0.6052 \times \text{cooked pH}) + (0.0072 \times \text{cooked ORP})}}}{1 + e^{(6.4154 - (0.6052 \times \text{cooked pH}) + (0.0072 \times \text{cooked ORP})}} \times 100
\]

\[
P(\text{pink}) = 100 - P(\text{no pink})
\]

The probability of pink color formation determined by the equation relates to the threshold values for individual breast muscle, which once exceeded may produce the pink color formation. The probability of pink color formation may be minimized by proper formulation of marinades to adjust the muscle pH down (more acidic) and increase oxidizing conditions (raise ORP). However, a formulation to minimize the probability of pink color may reduce yields, so a compromise formulation for acceptable yield and probability of pink color formation needs to be accessed. The separation of breast based on the three color groups: lighter than normal (“light”), normal (“normal”), and darker than normal (“dark”) (Fletcher, 1999; Fletcher et al., 2000) may allow a superior marinade to be formulated to obtain the greatest yield, while achieving a low probability of pink color formation because dark color breast are more susceptible to pink color formation than normal or light color breast, but produce a higher yield.

**Yields**

The yield of a product after cooking is possibly the most important factor to poultry processor because of monetary issues. The cooked yield and the process yield were measured for each of the four treatments of the normal and dark color breast groups. The cooked yield measures the amount of product retained between the marination and cooking procedures. The process yield measures the amount of product retained from the raw state of the breast throughout the cooking process.

The normal color breast group had lower cooked and process yields when compared to the dark color breast group (Figure 3.8). In the breakdown of the four treatments for normal color breast group, Treatment I produced significantly \((P < 0.05)\) lower cooked and process...
yields when compared to the other treatments, but no breast were found to have the pink defect in this treatment. Treatment III produced significantly ($P < 0.05$) higher cooked and process yields when compared to other treatments for the normal color breast group, but produced the highest number of breast with the pink color defect (11.11%). Treatment I and Treatment III were marinated with a sodium citrate/citric acid marinade with varying ionic strengths. However, Treatments II and IV were marinated with a sodium orthophosphate/sodium acid pyrophosphate marinade with varying ionic strength, but no differences in either the cooked or process yield were found. The increase in ionic strength may have an effect on Treatments I and III because of the lower marinade pH (5.7), but not on Treatments II and IV that were treated with the higher pH (6.0) marinade.

The dark color breast group had varying cooked and process yield between treatments. Treatments I and III had similar cooked and process yields, but were significantly lower than the yields for Treatments II an IV, which had similar yields. The treatments treated with the sodium citrate/citric acid marinade at the pH = 5.7 produced the lowest cooked and process yields, while the treatments treated with the sodium orthophosphate/sodium acid pyrophosphate marinade at the pH = 6.0 produced the highest cooked and process yields. The increased ionic strengths between treatments for the dark color breast group did not have an effect on the cooked or process yields. The difference in the yields for the dark color breast group may be a result of the increased pH of the marinade for Treatments II and IV or the different phosphate blend used in these treatments.

3.5 Conclusion

The factors associated with formation of the pink color defect in uncured fully chicken breast have been related to (1) preslaughter factors such as heat and cold stress, gaseous
environment, genetics, feed, hauling, and handling, (2) stunning techniques, (3) various classes and types of pigments, (4) current industry procedures including the use of nonmeat ingredients and cooking methods, (5) incidental nitrate/nitrite contamination through water supply, diet, freezing and processing equipment, and processing ingredients, and (6) irradiation of precooked products. The purpose of the current experiment was to discover ways to reduce the amount of pink color formation in uncured fully cooked chicken breast while maintaining an industry mindset. The result of the current research has revealed two possible theories to lessen the occurrence of the pink color defect as well as a prediction equation to determine the probability of pink color formation.

The use of a sodium citrate/citric acid marinade with a low ionic strength may reduce the occurrence of formation of the pink color defect in uncured, fully cooked chicken breast. Citric acid (Kieffer et al., 2000) reduced the pink defect in cooked ground turkey. A blend of sodium citrate and citric acid in combination with low ionic strength and a phosphate blend may allow the reduction of the pink color defect in intact breast meat. Yang and Chen (1993) reported citric acid decreases the external and internal CIE a* values (redness) of chicken breast. However, the low ionic strength of the marinade may reduce the cooked and process yield, so a compromise formulation may need to be found to satisfy yield requirements as well as occurrence of the pink color defect.

The occurrence of the pink color defect also may be related to a pH/ORP combination threshold. The treatments that exhibited the lowest amount of pink color defected samples had higher ORP value (less reducing conditions) with moderately high pH (6.2) values. The occurrence of the pink color defect is a given with an extremely low ORP (high reducing condition) or an extremely high pH (pH > 6.3), but there could possibly be a give and take
relationship between the pH and ORP. The threshold for pH and ORP will vary as either one of the two constituents increase or decrease. The raw and marinated pH and ORP need to be kept under control as well because the pink color formation may occur prior to cooking.

The prediction equation for the probability of pink color formation can be used to adjust the meat pH and ORP through marination to achieve a minimal probability of pink color formation.

\[
P(\text{no pink}) = \frac{e^{(6.4154 - (0.6052 \times \text{cooked pH}) + (0.0072 \times \text{cooked ORP}))}}{1 + e^{(6.4154 - (0.6052 \times \text{cooked pH}) + (0.0072 \times \text{cooked ORP})}}} \times 100
\]

\[
P(\text{pink}) = 100 - P(\text{no pink})
\]

However, the marinade formulation developed to minimize the probability of pink color needs to take the yield factor into account because chicken breast with no pink and low yields is not increasing profits for processors. The prediction equation can be used to provide a compromise marinade formulation to produce yield as well as an acceptable probability of pink color formation.

The superlative method for reducing the occurrence of the pink color defect would be to sort the breast based on the three color groups: lighter than normal (“light”), normal (“normal”), and darker than normal (“dark”) (Fletcher, 1999; Fletcher et al., 2000). The dark color breast muscle is more susceptible to formation of pink color because of it’s characteristically higher pH and ORP. Once the breasts were separated based on the color parameters, a marinade could be formulated using the pinking prediction equation to optimize the final cooked pH/ORP of the muscle to reduce pink color formation in dark color breasts and increase yields in lighter colored breast. The use of good manufacturing practices, such as using quality water free of
nitrates/nitrites throughout the plant, especially water used in marinade formulations, is a must to have a chance at successfully reducing the pink color defect.

**Table 3.6a:** Averages of chemical and physical measurements taken during the raw state, marinated state, and cooked state for the **normal color breast group**. Measurements were taken at each of the muscle phases during the experiment so that changes could be tracked. 1, 2, 3, 4, 5

<table>
<thead>
<tr>
<th></th>
<th>Treatment I</th>
<th>Treatment II</th>
<th>Treatment III</th>
<th>Treatment IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Pink</td>
<td>0</td>
<td>2.27</td>
<td>11.11</td>
<td>8.89</td>
</tr>
<tr>
<td>Raw muscle pH</td>
<td>pH = 5.7 u = 0.17</td>
<td>pH = 6.0 u = 0.21</td>
<td>pH = 5.7 u = 0.22</td>
<td>pH = 6.0 u = 0.25</td>
</tr>
<tr>
<td></td>
<td>6.01 ± 0.18</td>
<td>6.17 ± 0.20</td>
<td>6.11 ± 0.16</td>
<td>6.10 ± 0.17</td>
</tr>
<tr>
<td>Marinated muscle pH</td>
<td>6.08&lt;sub&gt;abcd&lt;/sub&gt; ± 0.13</td>
<td>6.16&lt;sub&gt;abcd&lt;/sub&gt; ± 0.20</td>
<td>6.14&lt;sub&gt;abcd&lt;/sub&gt; ± 0.13</td>
<td>6.11&lt;sub&gt;abcd&lt;/sub&gt; ± 0.10</td>
</tr>
<tr>
<td>Cooked muscle pH</td>
<td>6.24&lt;sub&gt;abc&lt;/sub&gt; ± 0.12</td>
<td>6.25&lt;sub&gt;abc&lt;/sub&gt; ± 0.17</td>
<td>6.22&lt;sub&gt;abcd&lt;/sub&gt; ± 0.11</td>
<td>6.18&lt;sub&gt;cd&lt;/sub&gt; ± 0.10</td>
</tr>
<tr>
<td>Marinated muscle ORP</td>
<td>- 25.54&lt;sub&gt;abcd&lt;/sub&gt; ± 24.22</td>
<td>- 31.92&lt;sub&gt;abcd&lt;/sub&gt; ± 18.23</td>
<td>- 37.73&lt;sub&gt;abc&lt;/sub&gt; ± 33.60</td>
<td>- 24.72&lt;sub&gt;abcd&lt;/sub&gt; ± 11.00</td>
</tr>
<tr>
<td>Cooked muscle ORP</td>
<td>- 78.57&lt;sub&gt;ab&lt;/sub&gt; ± 24.29</td>
<td>- 83.45&lt;sub&gt;ab&lt;/sub&gt; ± 19.90</td>
<td>- 101.21&lt;sub&gt;cd&lt;/sub&gt; ± 18.84</td>
<td>- 95.89&lt;sub&gt;cd&lt;/sub&gt; ± 15.64</td>
</tr>
<tr>
<td>Cooked CIE L* value</td>
<td>83.93&lt;sub&gt;abcd&lt;/sub&gt; ± 1.66</td>
<td>84.71&lt;sub&gt;abcd&lt;/sub&gt; ± 1.30</td>
<td>84.99&lt;sub&gt;abcd&lt;/sub&gt; ± 1.50</td>
<td>84.17&lt;sub&gt;abcd&lt;/sub&gt; ± 1.40</td>
</tr>
<tr>
<td>Cooked CIE a* value</td>
<td>2.56&lt;sub&gt;abc&lt;/sub&gt; ± 0.65</td>
<td>2.82&lt;sub&gt;abcd&lt;/sub&gt; ± 0.53</td>
<td>3.09&lt;sub&gt;abcd&lt;/sub&gt; ± 0.68</td>
<td>2.95&lt;sub&gt;abcd&lt;/sub&gt; ± 0.79</td>
</tr>
<tr>
<td>Cooked CIE b* value</td>
<td>11.79&lt;sub&gt;abcd&lt;/sub&gt; ± 1.45</td>
<td>11.53&lt;sub&gt;abcd&lt;/sub&gt; ± 0.97</td>
<td>12.10&lt;sub&gt;abcd&lt;/sub&gt; ± 1.11</td>
<td>12.19&lt;sub&gt;abcd&lt;/sub&gt; ± 1.00</td>
</tr>
<tr>
<td>% marinade pick up</td>
<td>12.06&lt;sub&gt;abcd&lt;/sub&gt; ± 2.69</td>
<td>11.44&lt;sub&gt;abcd&lt;/sub&gt; ± 2.90</td>
<td>11.78&lt;sub&gt;abcd&lt;/sub&gt; ± 3.13</td>
<td>11.82&lt;sub&gt;abcd&lt;/sub&gt; ± 3.77</td>
</tr>
<tr>
<td>% cooked yield</td>
<td>72.32&lt;sub&gt;a&lt;/sub&gt; ± 2.00</td>
<td>75.37&lt;sub&gt;ab&lt;/sub&gt; ± 1.82</td>
<td>77.29&lt;sub&gt;c&lt;/sub&gt; ± 2.93</td>
<td>75.95&lt;sub&gt;cd&lt;/sub&gt; ± 1.63</td>
</tr>
<tr>
<td>% process yield</td>
<td>81.02&lt;sub&gt;a&lt;/sub&gt; ± 2.15</td>
<td>83.97&lt;sub&gt;abcd&lt;/sub&gt; ± 2.08</td>
<td>86.36&lt;sub&gt;abcd&lt;/sub&gt; ± 3.14</td>
<td>84.89&lt;sub&gt;abcd&lt;/sub&gt; ± 2.26</td>
</tr>
</tbody>
</table>

1. Treatments with the same letters within each row are not significantly different base on an $\alpha=0.05$ significance level. Significant differences were only examined for marinated and cooked muscle states.
2. % Pink = the percentage of cooked chicken breast within each treatment that developed the pink color defect
3. CIE L* a* b* values were taken on the interior of the cooked chicken breast
4. Cook Yield = $\frac{\text{Cook weight}}{\text{Marinated weight}} \times 100$
5. Process Yield = $\frac{\text{Cook weight}}{\text{Raw weight}} \times 100$
6. The averages were calculated from the individual breast measurements within each treatment.
Table 3.6b: Averages of chemical and physical measurements taken during the raw state, marinated state, and cooked state for the dark color breast group. Measurements were taken at each of the muscle phases during the experiment so that changes could be tracked.  

<table>
<thead>
<tr>
<th></th>
<th>Treatment I</th>
<th>Treatment II</th>
<th>Treatment III</th>
<th>Treatment IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH = 5.7 u = 0.17</td>
<td>pH = 6.0 u = 0.21</td>
<td>pH = 5.7 u = 0.22</td>
<td>pH = 6.0 u = 0.25</td>
<td></td>
</tr>
<tr>
<td>% Pink</td>
<td>10.87</td>
<td>28.26</td>
<td>17.78</td>
<td>20.00</td>
</tr>
<tr>
<td>Raw muscle pH</td>
<td>6.23 ± 0.36</td>
<td>6.33 ± 0.22</td>
<td>6.28 ± 0.28</td>
<td>6.18 ± 0.20</td>
</tr>
<tr>
<td>Marinated muscle pH</td>
<td>6.26abc ± 0.32</td>
<td>6.21abc ± 0.19</td>
<td>6.22abc ± 0.23</td>
<td>6.31abc ± 0.17</td>
</tr>
<tr>
<td>Cooked muscle pH</td>
<td>6.32abc ± 0.22</td>
<td>6.32abc ± 0.21</td>
<td>6.32abc ± 0.17</td>
<td>6.23d ± 0.17</td>
</tr>
<tr>
<td>Raw muscle ORP</td>
<td>-16.30 ± 28.15</td>
<td>-36.40 ± 19.94</td>
<td>-45.83 ± 40.52</td>
<td>-35.20 ± 22.59</td>
</tr>
<tr>
<td>Marinated muscle ORP</td>
<td>-50.03abcd ± 31.15</td>
<td>-45.04abcd ± 15.06</td>
<td>-36.77abcd ± 39.04</td>
<td>-44.26abcd ± 13.55</td>
</tr>
<tr>
<td>Cooked muscle ORP</td>
<td>-113.90abcd ± 29.66</td>
<td>-109.00abcd ± 16.99</td>
<td>-109.36abcd ± 36.67</td>
<td>-112.52abcd ± 23.03</td>
</tr>
<tr>
<td>Cooked CIE L* value</td>
<td>83.02abcd ± 1.72</td>
<td>83.20abcd ± 1.79</td>
<td>83.32abcd ± 1.49</td>
<td>83.65abcd ± 1.67</td>
</tr>
<tr>
<td>Cooked CIE a* value</td>
<td>2.84abcd ± 0.78</td>
<td>3.43abc ± 1.17</td>
<td>3.20abcd ± 0.81</td>
<td>2.89abcd ± 1.05</td>
</tr>
<tr>
<td>Cooked CIE b* value</td>
<td>12.08abcd ± 0.99</td>
<td>11.23abc ± 1.13</td>
<td>11.72abcd ± 1.42</td>
<td>11.92abcd ± 1.36</td>
</tr>
<tr>
<td>% marinade pick up</td>
<td>12.17abcd ± 3.02</td>
<td>13.21abcd ± 2.67</td>
<td>12.45abcd ± 3.40</td>
<td>12.32abcd ± 2.90</td>
</tr>
<tr>
<td>% cooked yield</td>
<td>78.87abcd ± 2.21</td>
<td>80.71abcd ± 1.67</td>
<td>77.81abc ± 2.50</td>
<td>81.24abcd ± 1.47</td>
</tr>
<tr>
<td>% process yield</td>
<td>88.43ac ± 2.28</td>
<td>91.35bd ± 2.15</td>
<td>87.48ac ± 3.32</td>
<td>91.22bd ± 1.94</td>
</tr>
</tbody>
</table>

1. Treatments with the same letters within each row are not significantly different based on an $\alpha=0.05$ significance level. Significant differences were only examined for marinated and cooked muscle states.
2. % Pink = the percentage of cooked chicken breast within each treatment that developed the pink color defect
3. CIE L* a* b* values were taken on the interior of the cooked chicken breast
4. Cook Yield = \frac{Cook \ weight}{Marinated weight} \times 100
5. Process Yield = \frac{Cook \ weight}{Raw \ weight} \times 100
6. The averages were calculated from the individual breast measurements within each treatment.
Figure 3.1: Percent pinking for each marinade treatment within the cooked normal and dark color breast groups. The percent pinking is the percentage of cooked chicken breast within each treatment that developed the pink color defect.\(^1,2,3,4\)

1. Normal color raw breast = 47 < CIE L* < 50
2. Dark color raw breast = CIE L* < 47
3. Cooked breast with CIE a* ≥ 3.8 are considered pink (Pink Threshold)
4. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
Figure 3.2: Average percentage of marinade pick up during the marination phase of the experiment for the normal and dark color breast groups.  

1. Averages were calculated from individual breast marinade pick up within each treatment  
2. Treatment marinades were added to each batch of chicken breast at a 15% pump on a meat weight basis  
3. Each batch of chicken breast was vacuum tumbled for 20 minutes at 20 revolutions per minute with a 10 minute resting period after the tumble. A vacuum of 27 in. Hg was pulled for each tumble process  
4. % marinade pick up = \( \frac{\text{Marinated weight} - \text{Raw weight}}{\text{Raw weight}} \times 100 \)  
5. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
Figure 3.3: Average raw meat pH, marinated meat pH, and cooked meat pH for each marinade treatment within the normal and dark color breast groups. Measurements were taken at each muscle state so that the changes could be tracked.

1. Averages were calculated from individual breast measurements within each treatment
2. NC = normal color breast group
3. DC = dark color breast group
4. 1st and 2nd bars in the graph represent the average raw meat pH for the normal and dark color groups, respectively
5. 3rd and 4th bars in the graph represent the average marinated meat pH for the normal and dark color groups, respectively
6. 5th and 6th bars in the graph represent the average cooked meat pH for the normal and dark color groups, respectively
7. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
Figure 3.4: Average raw meat ORP, marinated meat ORP, and cooked meat ORP for each marinade treatment within the normal and dark color breast groups. Measurements were taken at each muscle state so that the changes could be tracked.  

1. ORP = Oxidation Reduction Potential  
2. Averages were calculated from individual breast measurements within each treatment  
3. NC = normal color breast group  
4. DC = dark color breast group  
5. 1st and 2nd bars within each treatment represent the average raw meat ORP for the normal and dark color groups, respectively  
6. 3rd and 4th bars within each treatment represent the average marinated meat ORP for the normal and dark color groups, respectively  
7. 5th and 6th bars within each treatment represent the average cooked meat ORP for the normal and dark color groups, respectively  
8. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
Figure 3.5: Average cooked ORP (top) and percentage of pinking (bottom) for each marinade treatments within the normal and dark breast groups. This figure shows the correlation between the percentage of pinking and the cooked ORP.  

1. Percent pinking = the percentage of cooked chicken breast within each treatment that developed the pink color defect.
2. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
Figure 3.6: Average cooked pH (top) vs. average cooked ORP (bottom) for each marinade treatments with the normal and dark color breast groups. The average values were calculated from the individual breast measurements taken at the cooked phase of the muscle.¹

¹. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
Figure 3.7a: Frequency histograms for individual breast cooked ORP values for each of the four marinade treatments of the normal color breast group. The individual ORP values in combination with individual cooked pH values (Figure 3.7c) express a threshold for developing pink color. Treatment I had individual breast cooked ORP values that were skewed toward a higher ORP when compared treatments that had more pink color development.¹,²,³,⁴,⁵,⁶

1. ORP = Oxidation Reduction Potential
2. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
3. Treatment I = 0% pinking
4. Treatment II = 2.27% pinking
5. Treatment III = 11.11% pinking
6. Treatment IV = 8.89% pinking
Figure 3.7b: Frequency histograms for individual breast cooked ORP values for each of the four marinade treatments of the dark color breast group. The individual ORP values in combination with individual cooked pH values (Figure 3.7d) express a threshold for developing pink color. Treatment I had individual breast cooked ORP values that were skewed toward a higher ORP when compared treatments that had more pink color development. \(^1,2,3,4,5,6\)

1. ORP = Oxidation Reduction Potential
2. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
3. Treatment I = 10.87% pinking
4. Treatment II = 28.26% pinking
5. Treatment III = 17.78% pinking
6. Treatment IV = 20.00% pinking
Figure 3.7c: Frequency histograms for individual breast cooked pH values for each of the four marinade treatments of the normal color breast group. The individual pH values in combination with individual cooked ORP values (Figure 3.7a) express a threshold for developing pink color. The treatments that developed less pink color had individual breast cooked pH values that were skewed towards a lower pH (6.0 – 6.1).1,2,3,4,5

1. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
2. Treatment I = 0% pinking
3. Treatment II = 2.27% pinking
4. Treatment III = 11.11% pinking
5. Treatment IV = 8.89% pinking
**Figure 3.7d:** Frequency histograms for individual breast cooked pH values for each of the four marinade treatments of the dark color breast group. The individual pH values in combination with individual cooked ORP values (Figure 3.7b) express a threshold for developing pink color. The treatments that developed less pink color had individual breast cooked pH values that were skewed towards a lower pH (6.0 – 6.1).1,2,3,4,5

1. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
2. Treatment I = 10.87% pinking
3. Treatment II = 28.26% pinking
4. Treatment III = 17.78% pinking
5. Treatment IV = 20.00% pinking
Figure 3.8: Average cooked and process yields for each marinade treatments within the normal and dark breast groups. The average yield values were calculated from the individual breast measurements.

1. 1st and 2nd bars within each treatment are the cooked yields for the normal and dark color groups, respectively.
2. 3rd and 4th bars within each treatment are the process yields for the normal and dark color groups, respectively.
3. Cooked Yield = \( \frac{\text{Cook weight}}{\text{Marinated weight}} \times 100 \)
4. Process Yield = \( \frac{\text{Cook weight}}{\text{Raw weight}} \times 100 \)
5. See Tables 3.1 through 3.4 (pp. 81-84) for treatment marinade formulation
3.6 References


Lawrie RA. 1966. Metabolic stresses which affect muscle. The physiology and biochemistry of muscle as food. EJ Briskey, RG Cassens, JC Trautman, ed. The University of Wisconsin Press, Madison, WI, p 137-64.


CHAPTER 4

QUALITY OF WATER USED IN POULTRY PLANTS

WITHIN THE STATE OF GEORGIA¹

4.1 Abstract

The quality of water being used in the poultry industry is causing economic loss and poor product quality due to water hardness and contamination with nitrates and nitrites. The yields of products are reduced by high water hardness levels because the binding sites within the muscle are bound to ions contained in the hard water. Nitrates/Nitrites present in water are one of the causes of “pinking” or the pink color defect found in poultry meat. As little as 1 ppm of nitrite in muscle will cause pink color formation in chicken breast muscle. Water being used in poultry plants across the State of Georgia was examined for the presents of nitrates/nitrites and for the water hardness levels. Results indicate that water treatment to reduce hardness and remove nitrates is needed is some poultry plants. Samples ranged from 0.1 ppm to 4.9 ppm nitrate in the seven plants tested.

INDEX WORDS     Nitrate, Nitrite, Water, Pinking, Georgia, Water hardness, Poultry
4.2 Introduction

The quality of water being used in the poultry industry is causing economic lost and poor product quality. There are many attributes that contribute to the quality of water being used in plants, which include water hardness and contamination with organic and inorganic constituents. Nitrates/Nitrites present in water being used in poultry processing plants is a cause of the “pinking” or pink color defect found in poultry meat. The pink color defect is a major problem that causes economic loss and customer dissatisfaction in uncured fully cooked poultry products. The poultry industry has been plagued by the pink color defect for many years, as well as to a lesser degree other meat industries. The color of meat is the most utilized quality characteristic used by consumers because it is easy to evaluate upon purchasing of the product (Schwarz et al., 1997). The freshly cut surface of a boneless skinless chicken breast is a common place for this pink defect to occur. There are also pink spots and pink rings associated with the pink color of the cut surface. This appears to consumers as an undercooked product and results in a complaints and commercial buyer discounting (Cornforth et al., 1986).

The “pinking” defect is very sporadic and years of research have been conducted to discover the cause of this defect and how to prevent this problem from occurring. Possible causes of this defect could be from preslaughter conditions, nitrates and nitrites contamination (Ahn and Maurer., 1987; Froning et al., 1969; Mugler et al., 1970; Nash et al., 1985), heat stability of cytochrome c (Girard et al., 1990), oven gases (Pool., 1956), and the formation of denatured globin and nicotinamide hemochromes (Cornforth et al., 1986). The contamination of meat with nitrites or nitric oxide from a wide variety of sources is the most generally accepted explanation. Injectors, smokehouses, stuffers, other processing equipment, water supply, and the diet of the birds are possible a sources of nitrate or nitrite contamination of meat (Cornforth et
The drinking water and feed of birds may lead to contamination with nitrates and nitrites as well as a pink color in the end product (Froning et al., 1967). Nitrogenous contamination can also originate from previously used processing equipment for curing and from water utilized for carcass chilling. The quality of processing water used in poultry plants from chilling to marinade formulation could cause the pink color defect to occur in uncured, fully cooked poultry products (Mugler et al., 1970).

Nitrites are a widely used additive in food and food systems, especially in the curing of meats. In cured meats, sodium nitrite is allowable to levels of 156 ppm in the United States. Sodium nitrite is the precursor to the formation of the pink cured meat pigment, mononitrosylhemochrome (Killday et al., 1988). Nitrite provides the characteristic pink color and flavor in cured meats and acts as an effective antibotulinal agent and preservative. In the curing of meat, nitrite has at least three functions. Firstly, mononitrosylhemochrome is produced by a reaction of nitrite with myoglobin to provide the characteristic pink color of cured meat. Secondly, nitrite contributes to the flavor profile of cured meat by inhibiting the development of rancid off-flavors. Also, nitrite prevents the growth of spoilage bacteria in the meat as well as pathogens, including Clostridium botulinum. Nitrates are also used in the curing of meats. Nitrate (NO₃) is broken down and reduced to nitrite (NO₂) by bacteria in anaerobic conditions, using the molybdopterin-containing nitrate reductase. Also, bacteria in the mouth and stomach can reduce dietary nitrate to nitrite (Nakamura and Nakamura, 1996).

The safety of nitrites in the diet is a concern. In high concentrations, nitrite is toxic to humans. Oxidation of oxyhemoglobin to ferrihemoglobin which leads to methemoglobinemia is the main toxic effect. This can cause “blue baby syndrome” in newborn infants because the methemoglobin reducing capacity is low and can be fatal (Nakamura and Nakamura, 1996).
There are a number of different sources of nitrate and nitrite in the diet. Vegetables are a major source along with contaminated drinking water, medicines, and sausages. For example, root vegetables such as potatoes contain 200 ppm nitrate and leaf vegetables such as lettuce contain 1000 ppm nitrate. A possible link between nitrite and cancer was established in the 1970s. In a wide range of animal species, N-nitroso compounds (nitrosamines) are carcinogenic and have been detected in cured meats after cooking (Cammack et al., 1999).

One major source of nitrates and nitrite found in water and vegetables is coming from surface leaching and run-off from farm applications. The amount of fertilizers, pesticides and imported animal feedstuffs being used is increasing to meet the demands for food production and to get high yielding crop varieties. The amount of Nitrogen containing fertilizers being used in the UK has increased sixfold since the 1950s (Hooda et al., 2000).

The main cause of surface water and ground water degradation can be contributed to excessive loss of nutrients from the soil as well as farm effluents in surface runoff and leaching (Hooda et al., 2000). Ground water discharges and sub-surface flow including tile or pipe drainage into streams from draining grassland and arable areas have contributed to large concentrations of nitrates in streams and other watercourses (Baker and Laflen, 1983; Hallberg, 1987; Gangbazo et al., 1995). The actual amount of nitrate in surface runoff is minimal, but large amounts of nitrogen contain compounds in runoff through nitrification and mineralization contributes to the total amount of nitrates found in ground water and water sources. The surface waters draining from arable farmland is usually significantly higher in nitrate concentrations than the runoff from livestock farming areas. This can be contributed to the nitrogen containing fertilizer used and to the nitrification and soil mineralization processes that occur during the
The plowing process after each crop or manure application. The intensity of agricultural production usually reflects on the amount of nitrate concentration found in that particular area.

There are two forms of inorganic nitrogen in the soil, which are nitrate and ammonium. As water moves through the soil, it is able to pick up nitrate ions because they are freely mobile below the rooting zone. The land use and management practices applied influence the extent of nitrate leaching. Also, the type of soil and its texture play an important role in the amount of leaching of nitrates that can occur (Hooda et al., 2000). The largest losses and greatest amount of leaching occurs in sandy and peat soils because of its loosely bound texture as compared to clay soil, which has a tighter packed texture and the smallest losses and least amount of nitrate leaching (Bergstrom and Johansson, 1991). The time of manure and fertilizer application can significantly increase the concentration of nitrates found in the soil and impact the amount of nitrates leaching into ground water. This is contributed to the amount of nitrogen the plant needs at the time of application. Plants require the most nitrogen during the spring months because of the growing season. Nitrogen is pulled up by the roots of a plant to be utilized for growth and energy. During the autumn and winter months the plants do not require as much nitrogen because they are not in a growth mode. The amount of manure and fertilizer applied to crops should be adjusted to meet the requirements of the plant to cut down on the amount of excess nitrogen introduced into the soil for leaching into ground water and to reduce the economical impact for the farmer (Bailey, 1993). August or September application of manure resulted in smaller nitrate losses when compared to an October or November application in research done by Froment et al (1992) and Smith and Chambers (1993).

As more and more nitrates and nitrites are being found in ground water and in water sources, poultry plants need to be aware of the quality of water being used in processing.
applications as well as sanitary applications. Ahn and Maurer (1989) reported that as little as 1 ppm nitrite ion causes pinking in turkey breast meat. Also, in sensory panels conducted by Heaton et al. (2000), pinkness was detected by trained panelist for pork shoulder, turkey breast, and chicken breast at sodium nitrite levels of 4 ppm, 2 ppm, and 1 ppm, respectively. Research has concluded that low levels of nitrite contamination is responsible for the pink color defect being found in uncured fully cooked poultry products. The use of reverse osmosis has been very effectively applied to water denitrification as shown by Schoeman and Steyn (2003). In their research, the nitrate concentration was reduced by reverse osmosis from 42.5 ppm to 0.9 ppm.

The quality of water being used in poultry plants could also be affecting the yields of further processed products. The yields of marinated products are being reduced by poor quality water. The hardness level of water being used in marinades is the main contributing factor. There is no satisfactory definition of the term water hardness, but it is used to describe the amount of alkaline earth ions present in water. The sum of the amount of calcium and magnesium present in the water is usually used to determine the water hardness level. These elements create several problems in industry by forming deposits of their carbonates on equipment (Capitan-Vallvey et al., 2003). The water hardness levels of water being used in marinade formulations should be reduced to decrease the amount of calcium and magnesium in the water and improve the overall water quality.

The removal of contaminants in water being used in poultry is a major step in improving the quality of products being produced. Reverse osmosis separates the components of a fluid performed by polymeric semi-permeable membranes through the application of pressure. The influent stream going into the reverse osmosis membrane is separated into two effluent streams: the “permeate”, which passes through the membrane and the “retentate” or “concentrate”, which
retains the solutes or suspended solids that have been rejected by the membrane. Most ions and organics are rejected by the reverse osmosis membranes depending upon the pore size of the membrane, which varies from 5 Å to 20 Å in diameter (Sourirajan and Takeshi, 1985). The use of reverse osmosis improves the quality of the water by removing virtually all undesirable taste, odor, and color causing solutes as well as produces water that is 99.9% bacteria free.

The objective of this study was to determine the water hardness levels and nitrate/nitrite concentrations in water used in poultry plants across the State of Georgia. The water used in plants may be a reason for reduced marination yields as well as be a cause of pink color formation in uncured, fully cooked chicken breast.

4.3 Materials and Methods

Water sampling

Water samples were taken from seven different poultry plants at various locations within the State of Georgia. The samples were taken at various times, from the winter months though the summer months. The samples were taken from water entering the plant before filtration, water that was used in chilling of the carcass, and the water used in marinade formulation. The samples were taken in small plastic bottles labeled with the plant name, date, and location of each sample. Samples were taken in triplicate to ensure a well representative sample was taken. The samples were transported back to the Food Science and Technology Extension department lab for collection. After all samples were taken from the various plants for the particular time period, they were taken to the University of Georgia Water Lab for analysis. This procedure was repeated for each time frame the samples were collected. The results of the analysis were bought back to the Extension lab for review.
Sample Analysis

The University of Georgia Water Lab preformed the analysis of the samples. In the analysis, water samples were tested for the water hardness level, nitrate/nitrite concentration, pH, and the amount of total dissolved solids. Inductively Coupled Plasma (ICP) and Cadmium Reduction methods were used by the lab for analysis of the water samples. Each sample experienced the same level of analysis to keep the samples consistent.

4.4 Results and Discussion

Nitrate Concentrations

The concentration of nitrates found in the processing water of poultry plant across the state of Georgia used in chilling and marinade formulation along with the incoming water source can be found in Table 4.1. The average nitrate concentration for all of the seven plants tested in this study was 1.4 ppm, which is above the concentration of nitrite required to cause pink color formation in fully cooked poultry products. If all of the nitrates found in the processing water were converted to nitrites by various means, this could be the cause of pink color formation in uncured fully cooked products. Of the samples taken, Plant 2 had the highest average concentration of nitrates in the incoming water source, chiller, and marination waters, which are 4.6 ppm, 4.9 ppm, and 4.9 ppm, respectively. Plant 7 had the lowest average concentration of nitrates in the marination water (0.1 ppm) and incoming water supply (0.1 ppm). Plant 3 had the lowest nitrate level for chiller water with an average concentration of 0.6 ppm. A graph of the nitrate concentration for all plants is presented in Figure 4.1.

The differences in the concentration of nitrates can be attributed to the location of the plant as well as the source of water. The plants tested had varying water sources, which included well water and city water. The city water was of better quality due to city filtration and
treatment. Also, the amount of agricultural practices neighboring the processing plant could impact the concentrations of nitrates being found in the water due to leaching and runoff of fertilizer applications. The location of the plant in the state could be a factor in the difference in concentration due to the different aquifers in which the water was taken.

**Nitrite Concentrations**

The concentrations of nitrites found in processing waters from all plants were minimal. The average concentration of nitrites for all seven plants used in the study was less than 0.02 ppm. All of the plants in this study had consistent nitrite concentrations of less that 0.02 ppm except for Plant 7, which had a nitrite concentration of 0.03 ppm for the incoming water source and 0.04 ppm for water used in marinade formulations. The quantity of nitrites in the processing waters of the seven plants was below the nitrite concentration threshold (1 ppm) for formation of the pink color defect in chicken breast muscle, but the nitrite concentration in combination with the amount of nitrates being converted to nitrite may push the total nitrite concentration over the threshold value and cause pink color formation.

**Seasonal Effects**

There was a significant seasonal effect of the concentration of nitrates found. The samples taken in the winter months had slightly higher nitrate concentrations than samples taken during the spring and summer months. This could be due to the amount of nitrogen used by plants in growth during the spring and summer months. As stated earlier, research done by Bailey (1993), shows that plants do not require as much fertilizer during the winter months because of slowed growth and the amount of fertilizer used should be adjusted to meet the requirements of the plants. The available nitrogen not used by plants during the dormant months could leach into the ground water and increase the nitrate concentration. This could be a
possible cause for elevated nitrate levels during the winter samples as compared to samples taken
from the exact same location during the spring and summer months.

**Water Hardness Levels**

The water hardness levels of processing water used in poultry plant across the State of
Georgia in chilling and marinade formulation along with the incoming water source can be found
in Table 4.2. The average water hardness level for all of the seven plants tested in this study was
78.9 ppm. Plant 2 had the highest average water hardness levels in chiller and marination
waters, which are 165.3 ppm and 150.1 ppm, respectively. Plant 6 had the highest water
hardness for incoming water with a level of 146.6 ppm. Plant 4 had the lowest average water
hardness levels for the incoming water supply (23.2 ppm) and marination water (23.6 ppm).
Plant 5 had the lowest water hardness level for chiller water with an average level of 36.5 ppm.
The differences in the water hardness levels can be attributed to the location of the plant as well
as the source of water being used. The plants tested had varying water sources, which included
well water and city water. The city water was of better quality due to city filtration and
treatment.

**4.5 Conclusion**

In conclusion, the quality of water used in poultry plants could affect the color and yields
of finished product. Of the plants tested in this study, the water quality needs improvement. A
suggestion for processors is to incorporate a high quality water filtration system or a reverse
osmosis system. The use of reverse osmosis systems has been shown to reduce the amount of
organic and inorganic constituents found in raw waters (Mohsen et al., 2003). However, the
water quality of the State of Georgia was quite good, but for use in marinades and in processing
of poultry products the removal of contaminants is recommended.
Table 4.1: Average nitrate concentrations measured in parts per million (ppm) for the incoming water source of each plant, water used in the chiller, and water used in marinade formulations for the seven plants sampled during this study.

<table>
<thead>
<tr>
<th>Plant Number</th>
<th>Incoming Water Supply</th>
<th>Chill Tank Water</th>
<th>Marinade Formulation Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant 1</td>
<td>0.5 ppm</td>
<td>n/a</td>
<td>0.6 ppm</td>
</tr>
<tr>
<td>Plant 2</td>
<td>4.6 ppm</td>
<td>4.9 ppm</td>
<td>4.9 ppm</td>
</tr>
<tr>
<td>Plant 3</td>
<td>0.6 ppm</td>
<td>0.6 ppm</td>
<td>n/a</td>
</tr>
<tr>
<td>Plant 4</td>
<td>0.3 ppm</td>
<td>n/a</td>
<td>0.3 ppm</td>
</tr>
<tr>
<td>Plant 5</td>
<td>0.9 ppm</td>
<td>1.1 ppm</td>
<td>1.0 ppm</td>
</tr>
<tr>
<td>Plant 6</td>
<td>0.7 ppm</td>
<td>n/a</td>
<td>0.7 ppm</td>
</tr>
<tr>
<td>Plant 7</td>
<td>0.1 ppm</td>
<td>n/a</td>
<td>0.1 ppm</td>
</tr>
</tbody>
</table>

1. n/a = sample location was not available within plant

Table 4.2: Average water hardness levels measured in parts per million (ppm) for the incoming water source of each plant, water used in the chiller, and water used in marinade formulations for the seven plants sampled during this study.

<table>
<thead>
<tr>
<th>Plant Number</th>
<th>Incoming Water Supply</th>
<th>Chill Tank Water</th>
<th>Marinade Formulation Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant 1</td>
<td>25.2 ppm</td>
<td>n/a</td>
<td>25.9 ppm</td>
</tr>
<tr>
<td>Plant 2</td>
<td>135.0 ppm</td>
<td>165.3 ppm</td>
<td>150.1 ppm</td>
</tr>
<tr>
<td>Plant 3</td>
<td>37.0 ppm</td>
<td>37.0 ppm</td>
<td>n/a</td>
</tr>
<tr>
<td>Plant 4</td>
<td>23.2 ppm</td>
<td>n/a</td>
<td>23.6 ppm</td>
</tr>
<tr>
<td>Plant 5</td>
<td>35.8 ppm</td>
<td>36.5 ppm</td>
<td>36.7 ppm</td>
</tr>
<tr>
<td>Plant 6</td>
<td>146.6 ppm</td>
<td>n/a</td>
<td>145.9 ppm</td>
</tr>
<tr>
<td>Plant 7</td>
<td>119.7 ppm</td>
<td>n/a</td>
<td>119.3 ppm</td>
</tr>
</tbody>
</table>

1. n/a = sample location was not available within plant
Research with this project has shown that as little as 1 ppm of NITRITE causes pinking in cooked poultry breast meat.

Figure 4.1: Average concentrations of nitrates measured in parts per million (ppm) for the incoming water source of each plant, water used in the chiller, and water used in marinade formulations for the seven plants sampled during this study.1

1. Missing data for chiller and marination water locations for some plants because location was not available for sampling.
4.6 References


Mohsen MS, Jaber JO, Afonso MD. 2003. Desalination of brackish water by nanofiltration and reverse osmosis. Desalination 157: 167 (Abstr.).


5.1 Conclusion

The factors associated with formation of the pink color defect in uncured fully chicken breast have been related to (1) preslaughter factors such as heat and cold stress, gaseous environment, genetics, feed, hauling, and handling, (2) stunning techniques, (3) various classes and types of pigments, (4) current industry procedures including the use of nonmeat ingredients and cooking methods, (5) incidental nitrate/nitrite contamination through water supply, diet, freezing and processing equipment, and processing ingredients, and (6) irradiation of precooked products. The purpose of the current experiments was to discover ways to reduce the amount of pink color formation in uncured fully cooked chicken breast while maintaining an industry mindset. The result of the current research has revealed two possible theories to lessen the occurrence of the pink color defect as well as a prediction equation to determine the probability of pink color formation and, a known fact, that water quality can cause poor quality products to be produced.

The use of a sodium citrate/citric acid marinade with a low ionic strength may reduce the occurrence of formation of the pink color defect in uncured, fully cooked chicken breast. A blend of sodium citrate and citric acid in combination with low ionic strength and a phosphate blend may allow the reduction of the pink color defect in intact breast meat. However, the low ionic strength of the marinade may reduce the cooked and process yield, so a compromise formulation may need to be found to satisfy yield requirements as well as occurrence of the pink color defect.

The occurrence of the pink color defect also may be related to a pH/ORP combination threshold. The treatments that exhibited the lowest amount of pink color defected samples had higher ORP value (less reducing conditions) with moderately high pH (6.2) values. The
occurrence of the pink color defect is a given with an extremely low ORP (high reducing condition) or an extremely high pH (pH > 6.3), but there could possibly be a give and take relationship between the pH and ORP. The threshold for pH and ORP will vary as either one of the two constituents increase or decrease. The raw and marinated pH and ORP need to be kept under control as well because the pink color formation may occur prior to cooking.

The prediction equation for the probability of pink color formation can be used to adjust the meat pH and ORP through marination to achieve a minimal probability of pink color formation.

\[
P (\text{no pink}) = \frac{e^{(6.4154 - (0.6052 \times \text{cooked pH}) + (0.0072 \times \text{cooked ORP}))}}{1 + e^{(6.4154 - (0.6052 \times \text{cooked pH}) + (0.0072 \times \text{cooked ORP})}}} \times 100
\]

\[
P (\text{pink}) = 100 - P (\text{no pink})
\]

However, the marinade formulation developed to minimize the probability of pink color needs to take the yield factor into account because chicken breast with no pink and low yields is not increasing profits for processors. The prediction equation can be used to provide a compromise marinade formulation to produce yield as well as an acceptable probability of pink color formation.

The superlative method for reducing the occurrence of the pink color defect would be to sort the breast based on the three color groups: lighter than normal (“light”), normal (“normal”), and darker than normal (“dark”). The dark color breast muscle is more susceptible to formation of pink color because of it’s characteristically higher pH and ORP. Once the breasts were separated based on the color parameters, a marinade could be formulated using the pinking prediction equation to optimize the final cooked pH/ORP of the muscle to reduce pink color formation in dark color breasts and increase yields in lighter colored breast.
The use of good manufacturing practices, such as using quality water free of nitrates/nitrites throughout the plant, especially water used in marinade formulations, is a must to have a chance at successfully reducing the pink color defect. The water tested in the current research throughout the State of Georgia showed that removal of contaminants is needed to produce products free of the pink color defect as well as improve yield of marination procedures. The present of 1 ppm concentration of nitrite in water supplies for poultry plants without filtration will cause an occurrence of the pink color defect.

In conclusion of the present research, a control of processes, procedures, and products is a must to reduce the occurrence of the pink color defect. The process and procedures used in operations should control factors associated with pink color formation to reduce the occurrence of the pink color defect. The water used in processes throughout the poultry plant, such as in chilling and marination, should be free of nitrates and nitrites as well as have a reduced water hardness level. The use of the pink predictability equation should provide suitable cooked pH and ORP values to successfully reduce the chance of pink color formation. Once the factors that contribute to pink color formation are controlled and other methods for pink color reduction are in practice, there should be a reduction in the occurrence of the pink color defect in chicken breast meat. However, further research is needed to effectively establish a pH/ORP threshold for pink color formation.
Appendix 3.1: Sample ionic strength calculation for marinade in muscle using the Treatment I marinade

Ingredients:
- Sodium Citrate = 33.33 g / 1000 ml
- Citric Acid = 7.0 g / 1000 ml
- Sodium Tripolyphosphate = 15.0 g / 1000 ml
- Tetrasodium Pyrophosphate = 5.0 g / 1000 ml
- Salt = 23.33 g / 1000 ml

Total Ingredient (Solids) = 83.66 g / 1000 ml
% water = 100-8.366 = 91.634 % water

*Marinade pH = 5.70
*15% pump (how much marinade being added into tumbler)
*76% water in chicken breast

*Ionic Strength (u) formula for each ionization = \[
\frac{\text{Co}}{10^{(pK-pH)}+1} = (H_2Ac)^{-1} + \text{H}^{+1}
\]

\[
\text{Co} = \frac{\text{(% Pick up) (g / 100 ml)}}{100\text{(% water in meat)} + \text{(% Pick up) (% water in marinade)}} \times 10 \times \frac{1}{\text{MW}}
\]

pK = dissociation constant for a compound (may have multiple ionizations)

Example ionization of a compound:
- H_3Ac \rightarrow (pK_1 = \text{first ionization dissociation constant}) \rightarrow (H_2Ac)^{-1} + \text{H}^{+1}
- (H_2Ac)^{-1} \rightarrow (pK_2 = \text{second ionization dissociation constant}) \rightarrow (HAc)^{-2} + \text{H}^{+1}
- (HAc)^{-2} \rightarrow (pK_3 = \text{third ionization dissociation constant}) \rightarrow (Ac)^{-3} + \text{H}^{+1}

Ionic strength calculation for Treatment I marinade in the muscle:

SODIUM CITRATE
*pK_1 = 3.14
*pK_2 = 5.95
*pK_3 = 6.39

1st ionization:

HOC(COONa)(CH_2COONa)_2 \rightarrow (pK_1 = 3.14) \rightarrow HOC(COO^{-1})(CH_2COONa)_2 + Na^{+1}

\[
\frac{\text{Co}}{10^{(pK_1-pH)}+1} = \frac{\text{Co}}{10^{(3.14-5.70)}+1} = \frac{\text{Co}}{1.002754} = 0.997 \text{ Co}
\]
Appendix 3.1: Continuation

2nd ionization:

\[
\text{HOC(COO}^{-1})(\text{CH}_2\text{COONa})_2 + \text{Na}^{+1} \rightarrow (\text{pK}_2 = 5.95) \quad \text{HOC(COO}^{-1})(\text{CH}_2\text{COO}^{-1})(\text{CH}_2\text{COO}) + \text{Na}^{+1}
\]

\[
\cdot \quad \frac{0.997 \text{ C}}{10^{-(\text{pK}_1 - \text{pH}) + 1}} = \frac{0.997 \text{ Co}}{10^{-(5.95 - 5.70) + 1}} = \frac{0.997 \text{ Co}}{2.778} = 0.359 \text{ Co}
\]

3rd ionization:

\[
\text{HOC(COO}^{-1})(\text{CH}_2\text{COONa})(\text{CH}_2\text{COO}^{-1}) + \text{Na}^{+1} \rightarrow (\text{pK}_3 = 6.39) \quad \text{HOC(COO}^{-1})(\text{CH}_2\text{COO}^{-1})(\text{CH}_2\text{COO}) + \text{Na}^{+1}
\]

\[
\cdot \quad \frac{0.359 \text{ C}}{10^{-(\text{pK}_1 - \text{pH}) + 1}} = \frac{0.359 \text{ Co}}{10^{-(6.39 - 5.70) + 1}} = \frac{0.359 \text{ Co}}{2.778} = 0.0609 \text{ Co}
\]

Amount of ions released for each ionization:

- 1st ionization = 0.997Co – 0.359Co = 0.638Co
- 2nd ionization = 0.359Co – 0.0609Co = 0.2981Co
- 3rd ionization = 0.0609Co – 0.00Co = 0.0609Co

Ionic Strength Determination for Sodium Citrate:

\[
u = 0.5 \text{ Co} \left[ 0.997 + 0.359 + 0.0609 + 0.638(\text{charge}^{-2}) + 0.2981(\text{charge}^{-2}) + 0.0609(\text{charge}^{-2}) \right]
\]

\[
u = 0.5 \text{ Co} \left[ 0.997 + 0.359 + 0.0609 + 0.638 (-1^{-2}) + 0.2981 (-2^{-2}) + 0.0609 (-3^{-2}) \right]
\]

\[
u = 0.5 \text{ Co} \left[ 3.7954 \right]
\]

\[
u = 1.8977 \text{ Co}
\]

\[
\text{Co} = \frac{\text{(% Pick up) (g / 100 ml)}}{100(\text{(% water in meat) + (% Pick up) (% water in marinade)})} x 10 \times \frac{1}{\text{MW}}
\]

\[
\text{Co} = \frac{(15)(3.333 \text{ g / 100 ml})}{100(0.76) + (15)(0.91634)} x 10 \times \frac{1}{258.06907}
\]

\[
\text{Co} = 0.02159
\]

\[
u = 1.8977 (0.02159) = 0.04097
\]
Appendix 3.1: Continuation

CITRIC ACID

MW = 192.13 amount = 7.0 g / 1000 ml

\[
Co = \frac{\text{(% Pick up) (g / 100 ml)}}{100(\% \text{ water in meat}) + \text{(% Pick up) (\% water in marinade)}} \times 10 \times \frac{1}{\text{MW}}
\]

\[
Co = \frac{(15) (0.70 \text{ g / 100 ml})}{100 (0.76) + (15) (0.91634)} \times 10 \times \frac{1}{192.13}
\]

\[
Co = 0.0061
\]

\[
u = 0.996 \text{ Co}
\]

\[
u = 0.996 (0.0061) = 0.00608
\]

SODIUM TRIPOLYPHOSPHATE

\[\text{Na}_5\text{P}_3\text{O}_{10}\]

MW = 367.93 amount = 15.0 g / 1000 ml

* pK\textsubscript{1} = ---
* pK\textsubscript{2} = 1.10
* pK\textsubscript{3} = 2.30
* pK\textsubscript{4} = 6.26
* pK\textsubscript{5} = 8.90

1\textsuperscript{st} ionization:

\[
\text{Co} = \frac{\text{Co}}{10^{(pK_{1} – \text{pH})} + 1} = \text{Co (no dissociation)}
\]

2\textsuperscript{nd} ionization:

\[
\text{Co} = \frac{\text{Co}}{10^{(1.10 – 5.70)} + 1} = \frac{\text{Co}}{1.0000} = \text{Co (no dissociation)}
\]

3\textsuperscript{rd} ionization:

\[
\text{Co} = \frac{\text{Co}}{10^{(2.30 – 5.70)} + 1} = \frac{\text{Co}}{1.0004} = 0.9996 \text{ Co}
\]
Appendix 3.1: Continuation

4\textsuperscript{th} ionization:

\[ \bullet = \frac{0.9996 \text{Co}}{10^{(6.26 - 5.70)} + 1} = \frac{0.9996 \text{ Co}}{4.6308} = 0.2159 \text{ Co} \]

5\textsuperscript{th} ionization:

\[ \bullet = \frac{0.2159 \text{ Co}}{10^{(8.30 - 5.70)} + 1} = \frac{0.2159 \text{ Co}}{1585.89} = 0.000136 \text{ Co} \]

Amount of ions released for each ionization:

- 1\textsuperscript{st} ionization = --------------- = Co (no dissociation)
- 2\textsuperscript{nd} ionization = 1.0000 Co – 0.9996 Co = 0.0004 Co
- 3\textsuperscript{rd} ionization = 0.9996 Co – 0.2159 Co = 0.7837 Co
- 4\textsuperscript{th} ionization = 0.2159 Co – 0.000136 Co = 0.2158 Co
- 5\textsuperscript{th} ionization = 0.000136 Co – 0.00 Co = 0.000136 Co

Ionic Strength Determination for Sodium Tripolyphosphate:

\[ u = 0.5 \text{ Co} \left[ 1.0 + 1.0 + 0.9996 + 0.2159 + 0.000136 + 0.00 \text{ (charge}^2\text{)} + 0.0004 \text{ (charge}^2\text{)} + \\ 0.7837 \text{ (charge}^2\text{)} + 0.2158 \text{ (charge}^2\text{)} + 0.000136 \text{ (charge}^2\text{)} \right] \]

\[ u = 0.5 \text{ Co} \left[ 3.215636 + 0.00 \text{ (-1}^2\text{)} + 0.0004 \text{ (-2}^2\text{)} + 0.7837 \text{ (-3}^2\text{)} + 0.2152 \text{ (-4}^2\text{)} + 0.000136 \text{ (-5}^2\text{)} \right] \]

\[ u = 0.5 \text{ Co} \left[ 13.7264636 \right] \]

\[ u = 6.8632 \text{ Co} \]

\[ \text{Co} = \frac{\text{(% Pick up) (g / 100 ml)}}{100(\text{% water in meat}) + (\text{% Pick up}) \text{(wtr in marinade)}} \times 10 \times \frac{1}{\text{MW}} \]

\[ \text{Co} = \frac{(15) \text{(1.50 g / 100 ml)}}{100 (0.76) + (15) (0.91634)} \times 10 \times \frac{1}{367.93} \]

\[ \text{Co} = 0.0068 \]

\[ u = 6.8632 \times 0.0068 = 0.04667 \]
Appendix 3.1: Continuation

TETRASODIUM PYROPHOSPHATE

Na₄P₂O₇

MW = 265.9  amount = 5.0 g / 1000 ml

* pK₁ = 0.85
* pK₂ = 1.49
* pK₃ = 5.77
* pK₄ = 8.22

1st ionization:

\[
\text{Co} = \frac{\text{Co}}{10^{0.85 - 5.70} + 1} = \frac{\text{Co}}{1.00001} = 0.999 \text{ Co}
\]

2nd ionization:

\[
\text{Co} = \frac{0.9999 \text{ Co}}{10^{1.49 - 5.70} + 1} = \frac{0.9999 \text{ Co}}{1.000061} = 0.9998 \text{ Co}
\]

3rd ionization:

\[
\text{Co} = \frac{0.9998 \text{ Co}}{10^{5.77 - 5.70} + 1} = \frac{0.9998 \text{ Co}}{2.1749} = 0.4597 \text{ Co}
\]

4th ionization:

\[
\text{Co} = \frac{0.4597 \text{ Co}}{10^{8.22 - 5.70} + 1} = \frac{0.4597 \text{ Co}}{332.13} = 0.001384 \text{ Co}
\]

Amount of ions released for each ionization:

- 1st ionization = 0.999 Co – 0.9998 Co = 0.0001 Co
- 2nd ionization = 0.9998 Co – 0.4597 Co = 0.5401 Co
- 3rd ionization = 0.4597 Co – 0.001384 Co = 0.4583 Co
- 4th ionization = 0.001384 Co – 0.00 Co = 0.001384 Co
Appendix 3.1: Continuation

Ionic Strength Determination for Tetrasodium Pyrophosphate:

\[
u = 0.5 \ Co \left[ 0.9999 + 0.9998 + 0.4597 + 0.001384 + 0.001384(\text{charge}^2) + 0.5401(\text{charge}^2) + 0.4583(\text{charge}^2) + 0.001384(\text{charge}^2) \right]
\]

\[
u = 0.5 \ Co \left[ 2.460784 + 0.0001 (-1^2) + 0.5401 (-2^2) + 0.4583 (-3^2) + 0.001384 (-4^2) \right]
\]

\[
u = 0.5 \ Co \left[ 8.768128 \right]
\]

\[
u = 4.3841 \ Co
\]

\[
Co = \frac{\left(15\right) \left(0.50 \text{ g / 100 ml}\right)}{100 \left(0.76\right) + \left(15\right) \left(0.91634\right)} \times 10 \times \frac{1}{265.9}
\]

\[
Co = 0.00314
\]

\[
u = 4.3841 \left(0.00314\right) = 0.0138
\]

SODIUM CHLORIDE (SALT)

NaCl \ MW = 58.44247

\[
Co = \frac{\left(15\right) \left(2.333 \text{ g / 100 ml}\right)}{100 \left(0.76\right) + \left(15\right) \left(0.91634\right)} \times 10 \times \frac{1}{58.44247}
\]

\[
Co = 0.0667
\]

\[
u = \frac{1}{Co}
\]

\[
u = 0.0667
\]
Appendix 3.1: Continuation

Total Ionic Strength for Treatment I Marinade in the Muscle:

- Sodium Citrate…………………u = 0.04097
- Citric Acid…………………u = 0.00608
- Sodium Tripolyphosphate……..u = 0.04667
- Tetrasodium Pyrophosphate………u = 0.0138
- Sodium Chloride……………u = 0.0667

Total ionic strength…………...u = 0.17422
u = 0.17

The total ionic strength within the muscle treated with Treatment I = 0.17

** This calculation procedure was used for all treatments using the pK’s listed below **

pK’s for ingredients:

Sodium Citrate: pK₁ = 3.14
pK₂ = 5.95
pK₃ = 6.39

Sodium Orthophosphate: pK₁ = 2.15
pK₂ = 7.09
pK₃ = 12.32

Tetrasodium Pyrophosphate: = pK₁ = 0.85
pK₂ = 1.49
pK₃ = 5.77
pK₄ = 8.22

Sodium Tripolyphosphate: = pK₁ = ----
pK₂ = 1.10
pK₃ = 2.30
pK₄ = 6.26
pK₅ = 8.90

Ionic Strength of Treatments:

- Treatment I  = 0.17  (pH = 5.7)
- Treatment II = 0.21  (pH = 6.0)
- Treatment III = 0.22  (pH = 5.7)
- Treatment IV = 0.25  (pH = 6.0)