# Simulation and Reproducibility of Pink Color Defect in Cooked Chicken Breast Meat

by

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(Under the direction of Manjeet S. Chinnan)

## Abstract

The pinking defect in cooked poultry white meat, which is associated with undercooking, results in serious economic losses to retailers, processors, and producers of such products. This well documented pink discoloration is attributed to specific in situ factors, such as pH, reducing conditions, and pigments' chemical state and reactivity. A simulation of the pink defect and the examination of quantifiable changes associated with it can aid in developing alternative processing methods to eliminate potential for pinking.

Samples were selected from three color groups (normal, lighter than normal, and darker than normal) of boneless, skinless, chicken breast muscles based on CIE L* color values. In situ changes were induced using sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite. The subjective pink threshold used in judging pink discoloration was established at CIE a* = 3.8. Muscles in all treatments were subjected to individual injections, followed by tumbling, cooking, and chilling. Both raw and cooked samples were analyzed for color (L*, a*, b*), reflectance spectra, pH, oxidation-reduction potential, and pigments.

Simulation of the pink defect was achieved in eight of the sixteen treatment combinations in the light group, nine in the normal group, and ten in the dark group. The simulation was possible both with and without presence of sodium nitrite. The presence of only 1 ppm of sodium nitrite produced significant pinking of cooked meat in all three color groups. Pinking was significantly affected by lightness (CIE L*) of raw muscles, increased pH and increased reducing conditions. Induced pH, oxidation-reduction potential, metmyoglobin concentration, and nitrosopigment content of raw meat affected the dark color group most and the muscles from the light group the least. The logistic regression demonstrated its feasibility of using raw meat conditions

for prediction of the pink defect. The model was able to account for more than 90% of variability using nitrosopigment, pH, and reducing conditions as the variables.

INDEX WORDS: Chicken white meat, Pink defect, Simulated pinking, Raw meat conditions, Cooked meat, Reproducibility of pinking

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*I dedicate this work to my parents, Apolonia and Jacek Wojciechowscy*
*in gratitude for their love, support, patience,*
*and confidence not only during my graduate studies*
*but throughout my life.*

*My heartfelt thanks go also to my husband, Andrzej,*
*for his understanding, and tolerant encouragement*
*during the time I have spent over 4,000 miles from home.*

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*Opportunity is missed by most people*
*because it is dressed in overalls, and looks like work.*
—Thomas A. Edison

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# Chapter 1

# Introduction

## 1.1 Statement of the problem

Color is a primary factor in public's acceptance of meat products. When we eat fully cooked poultry white meat we expect the hues to be off-white or gray. The U.S. Department of Agriculture, Food Safety and Inspection Service regulation requires that heat-processed poultry products sold as ready-to-eat must be cooked to 71.1°C (FSIS USDA, 1999). However, occasionally a pink discoloration in well-cooked white meats is found, which consumers may associate with undercooking. This problem in the industry is referred as "pinking", "pinkness", "pink defect" or "pink tinge". In this defect, white meat exhibits areas that retain a pink color even after the meat has been cooked to an internal temperature far above that required by USDA. As mentioned above, consumers may interpret the pink discoloration as an indicator of undercooked product which is unsafe to eat. This flaw causes dissatisfaction and eventually affects the purchasing behavior of consumers. Fast food establishments may overcook meat with pink defect because they commonly use color instead of an endpoint cooking temperature as criterion of doneness. In Georgia, about 1.25 billion broilers (USDA-NASS, 2002) are slaughtered and processed into food products each year. With such huge numbers, even a small fraction of defective product can translate into a very substantial economic loss for the poultry industry.

Considerable research, covering more than forty years, has focused on the appearance of pink coloration in cooked poultry. Pinking has been related to the presence of the following pigments: (1) undenatured myoglobin and oxymyoglobin, (2) nitrosyl hemochromes, the pink pigments of cured meat, (3) carbon monoxide hemochromes, (4) cytochrome c, and (5) globin hemochromes of well-cooked meats (Tappel, 1957; Bernofsky et al., 1959; Fox, 1966; Ledward, 1974; Izumi et al., 1982; Ahn and Maurer, 1987, 1989a,b, 1990; Trout, 1989; Girard et al., 1990; Cornforth et al., 1986, 1998; Ghorpade and Cornforth, 1993; Heaton et al., 2000; Nam and Ahn, 2002 ). Other

researchers focused on identifying sources of the external nitrate/nitrite contamination that causes the incidental pinking due to formation of nitrosopigment (Froning et al., 1968, 1969b; Mugler et al., 1970; Ahn and Maurer 1987; Fleming et al., 1991). In addition, a number of research papers evaluated the importance of processing factors such as genetics, feed, hauling and handling, heat and cold stress, gaseous environment, stunning techniques, nonmeat ingredients, cooking methods, endpoint cooking temperature, and irradiation (Froning and Hartung, 1967; Froning et al., 1969a, 1978; Janky and Froning, 1972; Babji et al., 1982; Ngoka and Froning, 1982; Ngoka et al, 1982; Sackett et al., 1986; Claus et al., 1994; Nam and Ahn, 2002; Young et al., 1996). The findings indicated that all such factors seem to influence the specific in situ conditions such as (1) chemical state of pigments, their structure and reactivity, (2) pH of the meat, (3) oxidation-reduction potential in the meat, and (4) presence of different endogenous compounds. Recently, several researchers have tried to reduce pinking using different non-pink generating ingredients (Schwarz et al., 1997, 1999; Slesinski et al., 2000a,b); their reports suggest that the ingredients' effectiveness may or may not depend on the raw meat conditions. The interactions of factors and mechanisms associated with the pink defect are complex; thus there is still a need to gain knowledge about how the industry could minimize or eliminate this defect. The present research attempted to simulate pink defect using ingredients that are commonly used by the industry and to study the relationship between these induced conditions and pinking. It is expected that these findings will help industry develop improved processing methods which will reduce or eliminate pink color defect in cooked poultry products. The specific objectives are presented in the following section.

## 1.2 Objectives

1. Simulation of the pink defect by modifying the in situ conditions of the meat using the ingredients commonly employed by the industry.
    - Establish a subjective pink threshold for judging the existence of pinking.
    - Examine the effects of treatments that induce significant changes in the color of raw and cooked meat.
2. Measure how changes in the in situ conditions of raw meat (pH, oxidation-reduction potential, etc.) caused by sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite are related to pinking.
3. Correlate the induced in situ conditions of raw meat to the occurrence of the pink defect in cooked meat using a binary logistic regression approach.
4. Examine the effect of treatments that induce quantifiable changes in the in situ conditions of cooked meat.
5. Identify cooked meat conditions that are associated with simulated pink defect.

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# Chapter 2

# Pink Color Defect in Poultry White Meat as Affected By Endogenous Conditions[1]

[1]K. Holownia, M. S. Chinnan, and A. E. Reynolds
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## 2.1 Abstract

The pinking defect in cooked, uncured meat has been a problem in the poultry industry for nearly 40 years. Through the years, research has suggested various processing factors that seem to influence the specific biochemical conditions (pH, redox potential, denaturation, reacting ligands) of the meat that are related to the chemical state of the pigments in cooked meat, their structure and reactivity. This review addresses endogenous conditions that affect the pigments' reactivity, and research studies conducted on in situ conditions resulting in pinking in cooked meat. Future studies could be devised for understanding mechanisms leading to developing processes for reduction/elimination of the pink defect in cooked white poultry meat.
*Keywords:* pinking, defect, poultry, cooked meat

## 2.2 Introduction

When the pink defect (pinking, pinkness, or pink tinge) of cooked poultry meat occurs, white meat exhibits areas that retain a pink color even after the meat has been cooked to an internal temperature far above that required by Food Safety and Inspection Service, United States Department of Agriculture. Although the pinking is a cosmetic problem, it can greatly affect the purchasing behavior of consumers. Consumers may interpret the pink discoloration as an indication of undercooked product which is unsafe to eat. Therefore, this defect can result in serious economic losses to the retailers, processors, and producers of poultry products. Froning (1995) presented an extensive review of many factors affecting poultry meat color and Maga (1994) reviewed the primary factors influencing pinking. Factors related to the pink defect include (1) various classes and types of pigments (Fox 1966; Tappel 1957; Ledward 1974; Livingston and Brown 1981; Izumi and others 1982; Cornforth and others 1986; Girard and others 1989; Ghorpade and Cornforth 1993), (2) preslaughter factors as genetics, feed, hauling and handling, heat and cold stress, and gaseous environment (Froning and Hartung 1967; Froning and others 1968a, 1969a, 1978; Babji and others 1982; Ngoka and others 1982; Sackett and others 1986), (3) stunning techniques (Ngoka and Froning 1982; Froning 1995; Young and others 1996b; Craig and others 1999), (4) incidental nitrate/nitrite contamination through diet, water supply, freezing and processing equipment, and processing ingredients (Froning and others 1968b,1969b; Mugler and others 1970; Nash and others 1985; Ahn and others 1987; Fleming and others 1991; Heaton and others 2000), (5) current industry procedures including the use of nonmeat ingredients and cooking methods (Pool 1956; Froning and others 1968c; Helmke and Froning 1971; Janky and Froning 1973; Trout 1989; Ahn and Maurer 1989a, 1990a,b; Claus and others 1994; Cornforth and others 1998), and recently, (6) irradiation of precooked products (Nam and Ahn 2002).

All of the mentioned factors seem to change the specific in situ conditions of the meat, including such characteristics as pH, reducing conditions, degree of denaturation, and reactivity of endogenous meat compounds. These in situ conditions affect the chemical state, structure, and reactivity of the pigments. Meat can be considered to be a light-scattering matrix of cellular material, myofibrillar proteins, connective tissue, and light-absorbing pigments (MacDougall 1970; Swatland 1983). Eventually the physical characteristics of meat such as its light scattering and absorbing properties are affected by the in situ condition and thereby influencing the final color of meat products. There is an extensive amount of knowledge and literature about pigments' chemistry and reactions. This review is an attempt to deal only with endogenous conditions that affect the pigments' reactivity and to present the research studies conducted through the years on in situ conditions which affect pinking in cooked white poultry meat.

**Pigments' denaturation and reactions with ligands**

The intensity of reflected light and hence the color and appearance of the meat are affected by a complex interaction between light scattering, selective absorbance of the pigments, and the sample structure (MacDougall 1970; Swatland 1983; Renerre 1990). Light absorption and color are consequences of both the resonance of the prosthetic heme and the reaction with binding molecules (MacDougal 1982). The chemistry of myoglobin and its reaction with ligands has been reviewed in general by Antonini and Brunori (1971) and with particular emphasis on meat color by Giddings (1977), Livingston and Brown (1981), and MacDougall (1982). All ferrous and ferric forms of myoglobin have six ligands bound to the iron cation. The heme pyrolle nitrogen occupies four of the six coordination positions. In the native myoglobin derivatives, the fifth positions is occupied by histidine. This leaves the sixth

position opened for substitution. The ability of a ligand to coordinate with the iron in native myoglobin is limited because of the small cleft in the protein structure; that is, steric hindrance exists for a large ligand (Livingston and Brown 1981). As a result, only small molecules (e.g. O, NO, CO) can bind to the iron center in the native myoglobin. Denaturation, the conversion of myoglobin and metmyoglobin to ferro- and ferrihemochromes respectively, is the process which involves the unfolding of the native myoglobin proteins. This process allows the exposure of the heme group to potential ligands allowing it to react with the iron center. Potential ligands can be formed when heat unfolds the polypeptide chains of proteins, causing exposure of active side chains of the amino acids (Brown and Tappel 1957; Ahn and Maurer 1990a). The heme groups, after denaturation of myoglobin, will combine with certain amino acids, denatured protein, and many other nitrogen-containing substances to produce hemochromes. The denatured globin hemochromes are characterized by many authors as (1) pinking pigments of well cooked meats refrigerated anaerobically, (2) a mixed complex of ferrous heme iron with nitrogen from nicotinamide or from amines of denatured proteins, (3) insoluble and not extractable, and (4) measured by highly characteristic spectral curves with reflectance minima at about 530, 555, and 415 nm (Pool 1956; Akoyunoglou and others 1963; Brown and Tappel 1957; Dymicky and others 1975; Cornforth and others 1986; Ghorpade and Cornforth 1993). The most likely hemochromes have as their non-heme constituent either denatured globin or nicotinamide or perhaps both (Brown and Tappel 1957). The fact that certain proteins have more affinity for hematin than others may be related to the spatial arrangement of the histidine residue in the protein. Histidine also has the ability to form stabilizing porphyrin-protein interaction via salt-linkages, hydrogen, and hydrophobic bonds (Ledward 1974). Dymicky and others (1975) studied color formation in cooked model systems. Formation of the color is related to the nature of the substituent and its position on the ring. The substituted carbonyl pyridines

produce a pink to reddish coloration, similar to that of the nitrite containing control. Nicotinic acid and esters in this study form tan, light pink complexes. Imidazole also form a stable pink pigment. The imidazole residues may be supplied by the histidine groups of the bound protein (Ledward 1974), the epsilon amine group of lysine, or the terminal alpha amino group of denatured protein. Histidine, lysine, and arginine all yield pink complexes with absorption peaks at ∼410 and ∼535 nm and shoulders at ∼580 and ∼620 nm that are spectrally similar to cooked meat hemoprotein (Ledward 1974). The added salt solubilizes myofibrillar proteins, and the heat increases the number of exposed reactable side chains, thereby providing more chances for heme-complex formation in the meat (Ahn and Maurer 1989a). According to Ahn and Maurer (1990a) histidine, cysteine, and methonine, or their side-chains from solubilized proteins and vitamin $B_6$ derivatives are very important factors in the pinkness of cooked meat. The pH range of 6.1 to 6.4 is critical for heme complex formation of myoglobin and hemoglobin with naturally present ligands such as histidine, cysteine, methionine, nicotinamide, and solubilized proteins (Ahn and Maurer 1990a). Pink color development associated with a protein such as globin, nicotinamides, or albumin is possible after heating and is dependent upon protein denaturation after cooking (Cornforth and others 1986). Claus and others (1994) studied the effects of cooking temperatures, chilling rate, and storage time relative to the formation of the pink, denatured globin-hemochromes complex. They concluded that in ground turkey breast meat containing 2% nicotinamide, sensory pinkness and CIE a* values are higher with an increasing endpoint temperature (71–80°C) and with a slower chilling rate of the cooked product. Nicotinamide may be superior in terms of hemochrome forming ability to any other substances tested because it readily reacts under reducing conditions in either neutral or basic pH solution (Brown and Tappel 1957). Formation of ferrohemochromes is greatly influenced by pH, ligand concentration and ligand electronegativity. The lower the electron-donor

power of the ligands the more the ferrous state becomes stable compared to the ferric state (Akoyunoglou and others 1963; Falk 1964). Increasing electronegativity apparently enhances reactivity, provided that steric factor do not prevail (Akoyunoglou and others 1963).

One of the nitrogenous groups reacting with heme to form hemochrome is nitric oxide which is used to preserve myoglobin in the ferrous state. Nitrates and nitrites are commonly used in cured products; even in uncured meat when a small but sufficient amount of nitrite is present in meat it will react and form pink nitrosopigment. The residual pink color may be caused by nitrate microbial conversion to nitrite to form a nitric oxide myoglobin. When a high amount of nitrate in meat and/or processing water and a long storage period are combined, the probability of the development of the pink color in the oven roasted turkey breast is very high (Nash and others 1985; Ahn and Maurer 1987). The addition of only 1 ppm nitrite causes a significant color change in oven roasted turkey breast (Ahn and Maurer 1989a). Processing ingredients such as dried soy isolates may contain a sufficient amount of nitrite to react with meat pigment (Heaton and others 2000).

Carbon monoxide binds tightly to myoglobin forming ferrous carboxymyoglobin with a visible spectrum similar to that of oxymyoglobin and nitric oxide myoglobin. Carboxymyoglobin provides a stable red color in meat even after the denaturation of proteins had occurred (Livingston and Brown 1981). Significant pinking of beef and turkey is related to carbon monoxide in gas ovens (Pool 1956; Cornforth and others 1998). Irradiation as a processing preservation technique may produce a sufficient amount of carboxymyoglobin to be responsible for the pink color formation (Nam and Ahn 2002). Nicotinamide and nitrite are popular among researchers to induce pink color in meat when studying the pinking phenomenon in white poultry meat (Schwarz and others 1997; Slesinski and others 2000a,b). Since the pink hemochromes are formed when certain ligands bind to the heme pigments, a novel approach was

presented to bind selected ligands to the heme ring that do not cause a pink color (Schwarz and others, 1997, 1999). Schwarz and others (1997) found the effective ligands (diethylenetriamine, ethylenedinitrilo-tetraacetic acid disodium salt, trans 1,2-diaminocyclohexane-N,N,N',N' tetraacetic acid monohydrate, calcium reduced nonfat dried milk) in the presence of sodium nitrite and nicotinamide, the pink color formation agents. Schwarz and others (1999) concluded that addition of 50 ppm of ethylenedinitrilo-tetraacetic acid disodium salt (EDTA) or diethylenetriamine pentaacetic acid (DTPA) to uncured turkey breast muscle was sufficient to reduce pinking after cooking. There is an opportunity for some dairy proteins or some components of dairy proteins to reduce or eliminate the pink defect (Slesinski and others 2000a,b), but the mechanism for the reduction is not yet established.

**Meat pH**

Muscle pH and meat color are highly correlated. Higher pH values are observed in dark muscle whereas low pH values are observed in light colored muscle (Fletcher 1999a). Since muscle pH is related to the biochemical state of the muscle at the time of slaughter and during rigor mortis development, the muscle pH influences both the light reflectance properties of the meat as well as the chemical reactions of the myoglobin. Most of the reactions which interconvert ferrous and ferric forms of myoglobin are reversible and set up a "dynamic" color cycle in meats (Fox 1966).

The rate and the extent of pH decrease during rigor mortis are influenced by intrinsic factors such as species, breed, muscle and animal variability, as well as extrinsic factors such as environmental temperature and degree of stress (Lawrie 1985). Sackett and others (1986) found that the adaptation of birds to stress caused by carbon dioxide exposure affects utilization of glycogen reserves, eventually causing increased pH (5.84–5.91) of the raw breast meat when compared to the pH (5.73)

of the control group. The redness of raw meat is also significantly higher in all meat from birds stressed through exposure to carbon monoxide prior to slaughter (Sackett and others 1986). Electrical stunning and postmortem stimulation have significant effect on the pH of raw meat and cooked meat; however, those differences seem to diminish after 24 h postmortem (Craig and others 1999). Even then, the problem of elevated pH influences early deboned poultry meat. According to Fletcher (1999 a,b), a strong relationship exists between chicken breast meat color and muscle pH with fillets from the darker groups having significantly higher pH values than the lighter groups. The lightness of cooked breast meat colors is more closely associated with raw breast meat pH than with cooked meat pH (Fletcher and others 2000).

At neutral pH for myoglobin and hemoglobin, the affinity of heme for the protein is very high. Under normal physiological conditions, the heme is quite well protected from damage by being buried in the protein's cleft (Livingston and Brown 1981). The absorption spectrum of deoxygenated myoglobin or hemoglobin is invariant over the pH range in which the pigment is stable (Antonini and Brunori 1971). The absorption of metmyoglobin is greatly influenced by higher pH, and, since myoglobin oxidizes readily, the influence of alkaline pH is important in spectrophotometric studies (Bowen 1949). The pigment reactions that are triggered by muscle pH also may contribute to the occurrence of the meat pink color defect.

Low pH muscle has an opened structure which allows for greater oxygen penetration. Structural changes in sarcoplasmic and myofibrillar proteins result in increase in the light-scattering power of the meat (MacDougall 1970). In meat with a high light scattering ability, such as low pH meat, light does not penetrate far into the meat before being scattered; hence there is relatively little absorption by myoglobin and so the meat appears pale and light in color. The instability of myoglobin at a pH lower than 6.0 is related to globin unfolding and to partial loss of helix formation (Janky and Froning 1973). The pH affects enzymatic activity of the mitochondrial

system, thereby altering the oxygen availability for heme reactivity (Livingston and Brown 1981; Renerre 1990). Myoglobin is less heat sensitive near its isoelectric point 7.0 than at lower pH values (Young and others 1996b; Ledward 1974). Janky and Froning (1972, 1973) studied the effects of pH and heat denaturation of turkey myoglobin. They found that myoglobin is more susceptible to denaturation when pH was $< 6.0$. Trout (1989) used a model system to show that the sensitivity of myoglobin to heat denaturation increases as muscle pH decreases. Autoxidation is accelerated by low pH (Livingston and Brown 1981; Gutzke and Trout 2002). Autoxidation rate increases with any conditions which destabilize the linkage of the heme to the protein moiety and lead to greater exposure of the heme.

High pH meat has a high water-binding capacity and is associated with greater translucence and less light scattering. This lower scattering allows greater light penetration and absorption which makes the meat appear darker and redder (MacDougall 1982; Bendall and Swatland 1988). At high pH ($\sim$6.1), the iron of heme is predominantly in the ferrous state, and low pH accelerates ferrous iron conversion to the ferric state (Ahn and Maurer 1990b). Controlling oxidation to metmyoglobin includes maintaining a high final pH (Livingston and Brown 1981) but, at the same time, allowing greater reactivity of heme pigments. The neutral and alkaline pH has a protective effect on the denaturation behavior of myoglobin. Increasing the pH from 5.5 to 7.0 markedly decreases the percentage of myoglobin denatured at a given temperature, except at a temperature where the myoglobin is completely denatured (Trout 1989). Young and others (1996a) suggested that cooking poultry meat before completion of the rigor process can lead to sufficient pH change such that the temperature required for myoglobin denaturation increases above that normally achieved. The presence of high levels of myoglobin or some of its redder forms due to incomplete denaturation during heat processing can account for poultry having a pink color similar to that of an undercooked product

(Trout 1989; Young and others 1996a). A study by Ahn and Maurer (1990b) showed that a pH>6.4 was favorable for the heme complex reactions of myoglobin and hemoglobin with most of naturally present ligands (histidine, cysteine, methionine, nicotinamide, solubilized proteins). At pH 7.4 and higher heme complex forming reactions are observed in a myoglobin solution with no additional ligands added. As the state of the pigment is largely decided by the meat pH, the reaction of the heme with other reactants is also affected. If the meat pH is higher than 6.3, the side chains of histidine, cysteine, and methonine from meat proteins, and nicotinamide start to form heme complexes and exhibit a pink coloration of meat.

Salts and phosphates increase the functional properties of meat proteins, and hence the functional properties of the meat products, by changing the conformation of the constituent proteins (Damodaran and Kinsella 1982). As a result of the change in conformation the proteins do not aggregate extensively when heated but form an ordered, three dimensional lattice structure (Siegel and Schmidt 1979). Salt increases the potential for myoglobin oxidation by decreasing the buffering capacity of meat, and by promoting lower oxygen tensions in meat (Seideman and others 1984). However, the combination of NaCl and phosphate generally has a beneficial synergistic effect on product color. Sodium chloride facilitates the release of structural proteins from the muscle cells at the surface of the meat during mechanical treatment. Phosphates may also cause slowing of the rate of pH decline. Together, the effects seem to be related to pH elevation, ionic strength, metal ion chelation, and structural interactions with myofibrillar proteins (Trout and Schmidt 1984; Li and others 2001). When treated with salt and sodium tripolyphosphate a significant increase in muscle pH occurs. The effect of cooking on pH however, depends on the aging time for the muscle (Young and others 1996a). According to Yang and Chen (1993), chicken meat is darkened significantly by the addition of phosphate, and the darkness was proportional to the amount of phosphate added. Alkaline phosphates

in particular have sufficient buffering capacity to counteract pH drop (Young and others 1999). Phosphates also reduce the rate of pigment and lipid oxidation by the chelation of free iron, a lipid prooxidant (Pearson and others 1977). This in turn would reduce oxidation of myoglobin because myoglobin, is also oxidized by lipid oxidation byproducts. Increasing the pH of fresh meat above 6.0 greatly reduces the rate of lipid and myoglobin oxidation (Yasosky and others 1984). A meat pH that is high enough to facilitate myoglobin reduction and decrease the susceptibility of both lipid and pigments to oxidation will consequently cause pink or reddish discoloration in cooked meats.

Meat protein functionality is attributed to changes in the electrostatic interactions between charged groups in the muscle protein. In addition, both autoxidation of myoglobin and denaturation of proteins are affected by pH. The denaturation is a prerequisite for forming hemochromes, the pigments of cooked meat. When myoglobin is denatured, several types of amino acids side chains can coordinate to heme iron. Most of the ligands which bind to myoglobin have an electronegative atom (N or O) that donates electrons to the metal (Tarladgis 1962; Livingston and Brown 1981). Electronegativity of the amino acids side chains (dissociation of hydrogen ion from a side chain) may be increased further by increased pH. Therefore, pH is one of the factors in heme complex formation with amino acids or protein ligands. With increased pH, ligands may donate electrons to ferrous iron and form pink stable ferrohemochromes (Akoyunoglou and others 1963; Ahn and Maurer 1990b).

**Reducing conditions**

An important factor in pink color formation is the oxidation-reduction potential (ORP) which determines the state of the hemochrome($Fe^{2+}$)/hemichrome($Fe^{3+}$) iron of globin hemochromes and is the determining factor in the ability of pigments to

combine with or tie up molecules. The ORP is a function of the pH and the presence of reductants. The high reducing conditions allow greater reactivity and interconversion of pigments that is not desirable from the point of meat color (Cornforth and others 1986; Ahn and Maurer 1989b, 1990b). The pink color intensity of the heme complexes formed from myoglobin and hemoglobin with naturally present ligands increases tremendously under reducing conditions (Ahn and Maurer 1990a). The oxidation-reduction potential tends to become more negative (reducing conditions) at a high pH (Antonini and Brunori 1971). The heme pigments can be reduced by a number of reductants, some of which are endogenous to muscle tissue. In the fresh meat the reducing substances endogenous to the tissue constantly reduce the oxidized pigment (Fox 1966). Meat reducing activity is related to the presence of intracellular nicotinamide coenzymes such as nicotinamide adenine dinucleotide (NAD+, reduced form NADH) and nicotinamide adenine dinucleotide phosphate (NADP+, reduced form NADPH) (Giddings 1974, 1977; Livingston and Brown 1981; Renerre 1990). The nicotinamide coenzymes (also known as pyridine nucleotides) are electron carriers. They play a vital role in a variety of enzyme catalyzed oxidation-reduction reactions. It is necessary to maintain mildly oxidizing conditions to prevent pink color defect in cooked meat under commercial conditions (Cornforth and others 1986; Dobson and Cornforth 1992). The in situ levels of reductants are important in controlling rates of metmyoglobin reduction to oxymyoglobin. Also, the nitrosylmetmyoglobin is formed in the presence of reductants (for example, cysteine or ascorbic acid) from nitric oxide and metmyoglobin; the nitrosylmetmyoglobin is reduced to nitrosomyoglobin in the presence of the same reductants (Livingston and Brown 1981). Moreover, whether ascorbate will act as an oxidant or a reductant toward myoglobin depends upon the ascorbate concentration and the presence of other redox compounds. When the redox potential range is from $-15$ mV to $-100$ mV, ground cooked turkey meat has been shown to exhibit two distinct absorption maxima,

the first one at 542 nm and the second at 580 nm (Ahn and Maurer 1989b). These two absorption maxima are the chromatic peaks of oxymyoglobin in the 500 – 600 nm wavelength range. A further decrease in its potential to −550 mV reduced the myoglobin fully to the ferrous state; only one peak was observed at 556 nm. The ORP of −550 mV or less causes heme iron to be in the ferrous state resulting in formation of only pink reduced hemochromes (Cornforth and others 1986). The oxidation-reduction potential is also affected by processing ingredients such as salt and phosphate. The redox changes could especially have strong effect on pinking in cooked turkey breast meat in the ORP range of +90 mV to −50 mV (Ahn and Maurer 1989b).

**The role of *cytochrome c***

An interesting aspect is the role of cytochrome c, the electron transport protein found in mitochondrium, in pink color defect formation in cooked white meat. Cytochrome c remains stable at heat levels that myoglobin cannot withstand and is therefore thought to be responsible for the residual pink color (Cornish and Froning 1974; Izumi and others 1982; Ahn and Maurer 1989b; Girard and others 1990). Native and stable cytochrome c may retain its natural color during heating of meat up to 105°C (Izumi and others 1982). Muscle with higher pH (∼6.6) contains more cytochrome c than muscle with lower pH (6.0–6.1)(Pikul and others 1986). Chilling methods (ice slush against air chilled) may influence cytochrome c content of chicken meat but no explanation is available (Fleming and others 1991). The reflectance spectra of dark raw breast muscle from birds exposed to excitement prior slaughter and from those allowed to struggle freely during slaughter indicated that the dark color may be due to higher concentration of cytochrome c (Ngoka and Froning 1982). The extinction coefficient of cytochrome c at 550 nm, which gives a pink red color, is

about threefold higher than that of myoglobin after reduction. Although the amount of myoglobin is 50-fold higher than that of cytochrome c in turkey breast meat, the contribution of cytochrome c to pinkness of breast meat could be higher than myoglobin in cooked meat (Ahn and Maurer 1989a,b). In addition, the autoxidation rate of turkey myoglobin exhibited a six fold slower rate in the presence of cytochrome c. The reduction of myoglobin is the probable cause of pinking in cooked meat since the undenatured cytochrome c remaining in meat after cooking is still active for electron transfer. The strong absorption peak at 414 nm, relative to other 520 nm and 550 nm remains even after cooking turkey breast samples at 75°C and 85°C. In terms of proportions and positions of the absorption, these three banded spectra correspond to the respective gamma, beta, and alpha bands, typical of cytochrome c in a low ferrous state. In this form, cytochrome c appears pink (Girard and others 1990). The heme complex forming reactions of cytochrome c are not affected greatly by pH, and the heme complexes formed by selected ligands with cytochrome c are more stable compared to those formed with myoglobin and hemoglobin (Ahn and Maurer 1990b). These differences may be associated with a different structure of cytochrome c compared to myoglobin and hemoglobin (Dickerson 1980). Moreover, degradation of mitochondria by cooking could have facilitated release of cytochrome c from inner membrane and therefore improved its extractability for reaction (Girard and others 1990). Lowering the redox potential from +130 mV to +30 mV could double the absorbance of purified cytochrome c at 550 nm (Ahn and Maurer 1989b).

**Efforts to prevent pinking in cooked poultry meat**

Good manufacturing practices concentrate on efforts to reduce or eliminate external contamination of nitrate and nitrite such that nitrosopigments would not significantly contribute to the pink discoloration. Cornforth and others (1986) also

proposed to create mildly oxidized conditions in meat products during processing to prevent formation of pink hemochromes after cooking. Nevertheless, these conditions may appear to be questionable in regard to the quality of the final product. When studying the pinking phenomenon in white poultry meat researchers attempt to use pink generating ligands such as nicotinamide and nitrite to induce pinking (Schwarz and others 1997, 1999; Slesinski and others 2000a,b). However, when the ligands are used in combinations with other ingredients it is not clear if pinking is directly related to pink generating ligands or the induced in situ conditions. Schwarz and others (1997, 1999) hypothesized that since hemochromes are formed when a specific ligand binds to the pigment, it may be possible, without generating a pink color, to bind such a ligand to the sixth coordinate position of heme iron. Several researchers attempted to reduce pinking using different ingredients, such as ligands binding to heme iron, without formation of pink color in cooked products (Schwarz and others 1997, 1999; Slesinski and others 2000a,b). In addition, there is an opportunity for some dairy proteins or some components of dairy proteins to reduce or eliminate the pink defect (Slesinski and others 2000a,b); however, the mechanism responsible for the reduction is not yet established. In addition, some of the ingredients examined in those studies are not yet approved to be used as non meat ingredients in the meat or poultry products.

The mechanism of pink color reduction is not yet established. Moreover, adding the non-pink generating ligands may or may not be effective depending on how the endogenous conditions of the raw meat were affected by processing procedures. Interactions of factors and mechanisms associated with the pink defect are complex. There is a need for research in the area of prediction of the pink defect based on conditions present in raw meat before cooking and during marination process. We believe that the simulation of the pink defect is essential as a tool to investigate procedures for its prevention and elimination. The industry has also expressed

a necessity for development of models for predicting the effects of different processing conditions on discoloration in the cooked products as evidenced by a project funded under the Georgia Traditional Industries Program (GA FoodPAC 2002).

## 2.3 Conclusions

The factors associated with pinking have been related to the presence of the different pigments' classes — such as undenatured myoglobin and oxymyoglobin, reduced ferrohemochromes, nitrosyl hemochromes, carbon monoxide hemochromes, and cytochrome c. The formation and chemical reactivity of these pigments are directly related to the in situ conditions that are represented by pH, redox potential, degree of denaturation, and presence of reacting ligands. Regardless of which native pigments or hemochromes are involved in the pink color defect, the means to control this problem have not been reported previously. Better understanding of how processing factors affect endogenous conditions of meat may lead to greater control over the mechanisms involved in the generation of the pink defect.

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# Chapter 3

## Evaluation of Induced Color Changes in Chicken Breast Meat During Simulation of Pink Color Defect[1]

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Submitted to *Poultry Science*

## 3.1 Abstract

The objective of the study was to establish a pink threshold and simulate the pink defect in cooked chicken breast meat with treatment combinations that would induce significant changes in the color of raw and cooked meat. The subjective pink threshold used in judging pink discoloration was established at a* = 3.8. Samples of three color groups (normal, lighter than normal, and darker than normal) of boneless, skinless chicken breast muscles were selected based on instrumental color values. The in situ changes were induced using sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite at two levels: present and not present. Fillets in all treatments were subjected to individual injections, followed by tumbling, cooking and chilling. Samples were analyzed for color (L*, a*, b*) and reflectance spectra. Simulation of the pink defect was achieved in eight of the sixteen treatment combinations when sodium nitrite was present and in an additional two treatment combinations when it was absent. Pinking in cooked samples was significantly ($P < 0.05$) affected by lightness (L*) of raw meat color. Results confirmed that it is possible to simulate the undesired pinking in cooked chicken white meat when in situ conditions were induced by sodium chloride, sodium tripolyphosphate, and sodium nitrite. The continuation of the simulation study can aid in developing alternative processing methods to eliminate potential pink defect.

(*Key Words:* pinking in poultry, chicken breast meat, simulation, pink defect, color)

## 3.2 Introduction

The poultry industry is troubled by several quality defects one of which is pinking (pinkness, pink tinge) of white meat in cooked products. In this defect, white poultry meat displays areas that retain a pinkish color even after the meat has been heated to an internal temperature exceeding 71.1°C, the temperature required by the Food Safety and Inspection Service, United States Department of Agriculture (FSIS USDA, 1999). Restaurants, particularly fast food establishments, commonly use meat color rather than end-point cooking temperature to indicate proper procedures and may overcook their product due to this defect. Consumers, expecting off-white and gray hues of an uncured cooked white meat, may consider pinking an indication of an undercooked product which is unsafe to eat. Even though food safety is not an issue in pink defect (No reports of illness have been associated with pinking in poultry), the defect may be responsible for substantial losses to the poultry industry due to rejection, rework, and condemnation. Factors related to pinking include (1) various classes and types of pigments (Brown and Tappel, 1957; Fox,1966; Ledward, 1974; Livingston and Brown, 1981; Izumi et al., 1982; Cornforth et al., 1986; Girard et al., 1989; Ghorpade and Cornforth, 1993), (2) preslaughter factors such as genetics, feed, hauling and handling, heat and cold stress, and gaseous environment (Froning and Hartung, 1967; Froning et al., 1968a, 1969a, 1978; Babji et al., 1982; Ngoka et al., 1982; Sackett et al., 1986), (3) stunning techniques (Ngoka and Froning, 1982; Froning, 1995; Young et al., 1996; Craig et al., 1999), (4) incidental nitrate/nitrite contamination through diet, water supply, freezing and processing equipment, and processing ingredients (Froning et al., 1969b; Mugler et al., 1970; Ahn and Maurer, 1987; Fleming et al., 1991), (5) current industry procedures including the use of nonmeat ingredients and cooking methods and endpoint cooking temperatures (Pool, 1956; Froning et al., 1968b;

Helmke and Froning, 1971; Janky and Froning, 1972,1973; Trout, 1989; Ahn and Maurer, 1989a,b, 1990a,b; Claus et al., 1994; Cornforth et al., 1998), and recently (6) irradiation of precooked products (Nam and Ahn, 2002). This well documented pink discoloration also may be attributed to specific in situ conditions such as pH, reducing conditions, the chemical state and reactivity of pigments, degree of denaturation, and reactivity of endogenous meat compounds.

Good manufacturing practices concentrate on efforts to reduce or eliminate external contamination by nitrate and nitrite such that nitrosopigments would not significantly contribute to the pink discoloration. When studying the pinking phenomenon in white poultry meat researchers make an attempt to use pink generating ligands such as nicotinamide and nitrite to induce pinking (Schwarz et al., 1997, 1999; Slesinski et al., 2000a,b). Schwarz and others (1997, 1999) hypothesized that since hemochromes are formed when a specific ligand binds to the pigment, it may be possible, without generating a pink color, to bind such a ligand to the sixth coordinate position of heme iron. In addition, there is an opportunity for some dairy proteins or some components of dairy proteins to reduce or eliminate the pink defect (Slesinski et al., 2000a,b). However, the mechanism responsible for this reduction is not yet established.

Interactions of factors and mechanisms associated with the pink defect are complex. We believe that the simulation of the pink defect is an essential tool to investigate procedures for its prevention and elimination. For that reason, a study was initiated to simulate the defect in the intact breast muscles. This defect was simulated by modifying the in situ conditions using the ingredients that are commonly used by the meat industry. Therefore, the specific objectives involve (1) establishing a subjective pink threshold for judging the existence of pinking, and (2) examining the effects of treatments that induce significant changes in the color of raw and cooked meat.

## 3.3 Materials and Methods

### Sample Collection and Preparation

Boneless, skinless, chicken fillets (*Pectoralis major*) were obtained from two commercial processing plants. Breast fillets were selected based on three color groups: lighter than normal ("light"), normal ("normal"), and darker than normal ("dark") (Fletcher, 1999; Fletcher et al., 2000). Fillets were first sorted based on visual appearance at the deboning line or at the beginning of a "further processing" line. Sorting was verified using Hunter Lab reflectance colorimeter[2]; fillets were selected based on the medial surface (bone side) lightness (L*) values. L* values for the three color groups were: $L^* < 47$ for the darker than normal group ("dark"), $47 < L^* < 50$ for the normal group ("normal"), and $L^* > 50$ for the lighter than normal group ("light"). The samples were then bagged according to color group (light, normal, and dark), packed with ice, and transported to the laboratory.

The experiment was repeated twice for each of the two processing plants. To introduce seasonal variation in the material, each sampling for replication was separated by a period of at least 4 months. Two hundred forty fillets (eighty for each of the three color groups) were collected for each replication per plant resulting into a total of 960 product samples. All 240 samples associated with each replication and plant could not be collected in a single visit due to unavailability of the desired number of breast fillets (80) in each color group at a given time. Thus two or three visits were made as needed.

All breast fillets, 24 h postmortem, were vacuum packed using an Ultravac packaging machine[3] into sampling polyethylene bags, labelled and coded individually, then frozen and stored at $-18°C$ for up to 3 weeks before the experiment. After

[2]Miniscan/XE 45/0-L, Hunter Associates Laboratory, Inc., Reston VA 22090

[3]ULTRAVAC 250 KOCH Packaging, Division of KOCH Supplies, Inc., Kansas City, MO 64108

adequate thawing at 4–5°C (approximately 10–12 h) samples were ready for study. There were four treatment factors, namely sodium chloride[4] (1g/100g meat), sodium tripolyphosphate[5] (0.5g/100g meat), sodium erythorbate[6] (0.0546g/100g meat), and sodium nitrite[7](1ppm). Two levels of factors were selected: present and not present. Therefore, sixteen treatment combinations were used. The level of each factor was chosen according to standard regulations and common industry applications. The injection method (12% of meat weight basis) was used to incorporate ingredients into the samples. The ingredients were prepared in stock solutions: 0.1% of sodium nitrite, 10% of sodium tripolyphosphate, and 1% of sodium erythorbate. Next, the stock solutions were combined to obtain the final volume required for each sample. Finally, according to the weight of a given sample, sodium chloride was added as dry ingredient to the injection solution. Separate volumes of injection solutions were prepared and injected. Sixty-four injection solution were required (4 muscles x 16 treatment combinations). Uniformly-spaced multiple injections were carried out with a 35 ml syringe[8] equipped with a stainless steel 16 hole spray needle[9]. Once injected, samples were packed individually in moisture-impermeable polyethylene bags and closed, tumbled for 15 min with ice and refrigerated for equilibration overnight (12 h) at 4–5°C before further experimentation.

The subjective pink threshold used in judging the pink discoloration was produced by injecting samples from the 'normal' group with a solution containing sodium chloride and sodium nitrite to obtain a final concentration of 1, 2, 3, 4, and 5 ppm in the sample. The stock and injection solutions were prepared as explained above.

[4]Catalog No. S271-3, Fisher Scientific, www.fishersci.com
[5]Catalog No. T-5883, SIGMA CHEMICAL CO., St. Louis, MO 63178
[6]Catalog No. 49,633-2, Aldrich Chemical Company, Inc., Milwaukee, WI 53233
[7]Catalog No. S-2252, SIGMA CHEMICAL CO., St. Louis, MO 63178
[8]MONOJECT 35cc Syringe, Sherwood Medical Company, St. Louis, MO 63103
[9]MONOJECT 35cc Syringe, Sherwood Medical Company, St. Louis, MO 63103

The tumbling process was done in a laboratory made small table-top tumbler (O.D 40 cm, two equally spaced webs). Each time, four individually packed muscles (∼25% load) from the same treatment were tumbled for 15 min at 45 RPM with 1.0 kg of ice in order to keep the product temperature at about 0°C.

The following morning, two fillets of four from the experimental unit were designated for raw analysis, and the other two were subjected to cooking. The two fillets were placed in aluminum mini broiler pans[10] and covered with aluminum foil. Samples were cooked in a 167°C convection oven[11] to an internal temperature of 74°C, then cooled to 48°C within first 45 minutes in a temperature controlled laboratory where the temperature was maintained constant at 21°C. Following that the samples were cooled to 4.4°C by placing the samples in the walk-in environmental cooler maintained at 4°C. The final targeted temperature of the samples (∼4°C) was achieved within 4 h. Internal temperature of the product was monitored throughout the cooking and cooling process using K-type chromel-alumel thermocouples inserted in the center of a cranial end of a muscle before cooking. One thermocouple was used per cooking pan and eight pansfull (eight treatments with duplicate samples) were cooked at a time. HP BenchLink Data Logger 1.1 and data acquisition unit[12] was used for data collection.

**Color Evaluation**

Color was objectively evaluated, using reflectance spectrophotometry[2] (Large Area View, 25 mm aperture with Illuminant D65-daylight, 10° Standard Observer, wavelength of 400–700 nm with 1.0 nm accuracy), for reflectance spectra and colorimetric

[10]Mini Broiler Pans Size: 20 cm x 16.2 cm x 3.3 cm, HANDI-FOIL Corporation, Wheeling, IL 60090

[11]STABIL-THERM Lindberg/Blue M Electric Oven, GS LINDBERG/BLUE M, Blue Island, IL 60406

[12]HP 34970A Data Acquisition/Switch Unit with 20 Channel HP 34901A Multiplexer, Hewlett-Packard Company, Loveland, CO 80537

CIE (1978) L*, a*, and b* (CIE, 1978). Cooked samples were viewed using a light booth[13] (D65 as the Illuminant source). The raw and cooked samples were sliced into halves, lengthwise horizontally. Immediately, color was visually appraised, and color measurements taken. Four color measurements were taken on each half of the fillet; two readings on the anterior and two on the posterior portion of the muscle, rotating the samples 90° between measurements. The average value of the eight measurements (four per half) was subjected to statistical analysis. Chroma and hue angle were calculated based on average a* and b* values to further explain color change between samples:

$$Chroma, C = \sqrt{(a^*)^2 + (b^*)^2}$$

$$Hue\ angle, h^\circ = tan^{-1}\left(\frac{b^*}{a^*}\right)$$

The reflectance data at each wavelength was averaged by treatment and converted into a graph using an Excel program (Microsoft Excel 2000).

**Statistical Analyses**

The experiment was a partially confounded $2^4$ factorial design. Only eight of the sixteen treatments could be evaluated per day; therefore, the experiment was arranged into two blocks, thus introducing a confounding effect. The plant, replication, and treatments effects for L*, a*, b* color values were analyzed using the ANOVA option of the general linear models (GLM) procedures of SAS (SAS Institute, 1989). All possible fifteen orthogonal contrasts (treatment combinations) were analyzed and estimated. Planned comparisons between treatments were done using the LSD method only if ANOVA F-test and corresponding contrasts were significant at $P < 0.05$ (Fisher protected LSD). Otherwise, planned comparisons with the Bonferroni method ("data snooping") was performed (lsmeans option with PROG GLM

[13]byko-spectra 1 CG-6050, BYK-Gardner, Columbia, MD 21046

procedure of SAS). The three tested color groups in the experiment were subjected to individual statistical analyses.

## 3.4 Results and Discussion

### Subjective Pink Threshold

Establishment of a subjective pink threshold was an important step towards setting a cutoff point for judging the existence of pinking during the experiment. Table 3.1 presents instrumental color values of the cooked samples examined to establish the pink threshold. Visual appraisal of the cooked samples in the light booth showed all the samples injected with nitrite to have pinkish discoloration. Samples injected with only 1 ppm of sodium nitrite had a light pinkish discoloration that was distinguishable from a control sample injected with sodium chloride only. Samples injected with 1 ppm sodium nitrite and 1% sodium chloride were compared to the controls injected with 1% sodium chloride; this comparison resulted in establishing the least discoverable visible pinking in cooked samples. During the six replications the a* value ranged from 3.79 to 3.85 for the 1 ppm level. Statistical analysis showed no significance ($P > 0.05$) for L* and b* between samples injected with different sodium nitrite levels (with the exception of the control sample). The a* value of the sample injected with 1 ppm of nitrite differed from those injected with 3, 4, and 5 ppm but not from that injected with 2 ppm. In samples injected with 4 ppm and 5 ppm a* values were significantly different from the rest of the samples. The change in a* value was accompanied by change in hue angle which decreased from 72.86° in 1 ppm to 67.05° in 5 ppm sample. Chroma values were similar for all the pink samples and ranged from 12.26 to 13.12. The highest chroma value (16.13) and hue angle (80.65°) were found in the control sample. The results indicate that among pink samples, the a* value, but not L* or b* values, were affected by nitrite. Thus, when chroma values

Table 3.1: Mean (±SEM) CIE lightness (L*), a*, and b* color parameters of cooked breast fillets in pink threshold evaluation[1]

| Sodium nitrite concentration (ppm) | L* | a* | b* |
|---|---|---|---|
| 0 (Control) | 81.17 ± 0.28 | 2.62 ± 0.37$^{d}$ | 15.92 ± 0.53$^{a}$ |
| 1 | 81.91 ± 0.27 | 3.81 ± 0.02$^{c}$ | 12.36 ± 0.34$^{b}$ |
| 2 | 82.26 ± 0.27 | 4.01 ± 0.15$^{bc}$ | 12.50 ± 0.71$^{b}$ |
| 3 | 80.98 ± 0.74 | 4.38 ± 0.16$^{b}$ | 11.74 ± 0.59$^{b}$ |
| 4 | 80.01 ± 0.04 | 4.71 ± 0.19$^{a}$ | 11.32 ± 0.28$^{b}$ |
| 5 | 80.07 ± 0.06 | 4.94 ± 0.34$^{a}$ | 11.67 ± 0.71$^{b}$ |

n = 6
[1]Normal color group muscles
$^{a-d}$Means within a column with no common superscript differ significantly ($P < 0.05$)

were similar, a* value seemed to be causing the most changes in hue angle. Therefore, a* is the best choice to use as the threshold value within the range 79 – 84 for L* value and 10 – 16 for b* value. Previous research (Heaton et al., 2000) had also confirmed that the a* value correlates well with sensory (visual) scores of meat color. A comparison of results from a visual examination and colorimetric data established the least visible pinking at a* = 3.8. This value represents an average value from six replications. The pink threshold presented here is even lower than the a* values of > 4.0 for white meat reported by other authors (Ahn and Maurer, 1989b; Heaton et al., 2000).

## Colorimetric Analysis

**Significance of Plant and Replication.** The results of the combined ANOVA of raw and cooked meat for replication, plant, and contrasts' effects in color

measurement are presented in Tables 3.2, 3.3, and 3.4 for light, normal, and dark group, respectively. The replication effect within each plant was originally tested using a 'block within plant' effect as a divisor in a confounding design. The most variation within-plant was found in the light group where replication was significant ($P < 0.05$) for both plants with regard to lightness (L*) in raw samples. In addition, the second plant showed significance of replication for a* and b* in raw, and L* in cooked samples. There was only a significant replication effect in the second plant when L* of cooked samples was analyzed for the normal group. The dark group showed no significant effect of replication in either plant.

The results indicated that some variability exists, especially in light muscles, even within a single plant. Testing the plant-effect using residual error as a divisor showed no significance ($P > 0.05$) for CIE L*a*b* color of raw or cooked samples. Thus, in subsequent analyses the data were pooled across plants and replications.

**Color of Raw Samples.** The lightness (L*) values of raw samples for the light, normal, and dark groups were significantly affected by 1% of sodium chloride, showing decreases in value from 63.33 to 54.57, from 56.03 to 50.52, and from 50.94 to 38.02, respectively. The presence of sodium tripolyphosphate in injection solutions produced a significant ($P < 0.05$) negative effect on L* value for light and normal groups, but not for the dark group. The a* value of the light and dark groups also was affected by sodium chloride. In the light and normal groups, a significant positive effect on a* value occurred for sodium nitrite when it was injected alone or in combination with other ingredients. The a* values ranged from 7.82 to 10.24 vs. 5.45 for the light control group, and from 8.71 to 11.88 vs. 7.81 for the normal control group. Thus, the presence of nitrite resulted in direct formation (without oxidation to metmyoglobin first) of red appearing nitric oxide myoglobin. The use of intact muscle for the simulation probably allowed the reducing activity of the breast muscles to be conserved. Values of b* of the raw samples in the dark group were

Table 3.2: Analysis of variance table (F-statistic; P-probabilities) of CIE color parameters (L*, a*, b*) for replication (within each plant), plant, and treatment contrasts' effect in the light group of raw and cooked samples

| Source | Statistic | *Raw* | | | *Cooked* | | |
|---|---|---|---|---|---|---|---|
| | | L* | a* | b* | L* | a* | b* |
| Replication in: | | | | | | | |
| Plant 1 | F | 116.70 | 0.03 | 4.20 | 3.85 | 0.10 | 11.35 |
| | P | 0.0085 | 0.8736 | 0.1769 | 0.1889 | 0.7835 | 0.0780 |
| Plant 2 | F | 19.12 | 295.91 | 45.72 | 29.27 | 1.86 | 5.81 |
| | P | 0.0485 | 0.0034 | 0.0212 | 0.0325 | 0.3062 | 0.1375 |
| Plant | F | 0.13 | 0.75 | 1.13 | 0.62 | 0.19 | 0.01 |
| | P | 0.7575 | 0.4776 | 0.3996 | 0.5134 | 0.7085 | 0.9404 |
| Treatments[1] | | | | | | | |
| S | F | 58.40 | 8.56 | 3.71 | 9.56 | 0.01 | 8.10 |
| | P | 0.0001 | 0.0053 | 0.0602 | 0.0033 | 0.9301 | 0.0065 |
| P | F | 8.83 | 0.00 | 1.80 | 0.74 | 0.18 | 2.25 |
| | P | 0.0047 | 0.9936 | 0.1859 | 0.3932 | 0.6712 | 0.1403 |
| SP | F | 3.75 | 0.71 | 0.01 | 13.24 | 1.14 | 1.22 |
| | P | 0.0588 | 0.4030 | 0.9210 | 0.0007 | 0.2906 | 0.2746 |
| E | F | 0.09 | 0.00 | 0.00 | 0.41 | 0.02 | 0.11 |
| | P | 0.7671 | 0.9554 | 0.9657 | 0.5275 | 0.8838 | 0.7426 |
| SE | F | 0.00 | 0.01 | 1.31 | 0.04 | 0.07 | 0.05 |
| | P | 0.9770 | 0.9291 | 0.2583 | 0.5444 | 0.7961 | 0.8314 |
| PE | F | 1.08 | 0.10 | 0.05 | 0.22 | 0.05 | 0.11 |
| | P | 0.3034 | 0.7591 | 0.8271 | 0.6386 | 0.8239 | 0.7453 |
| SPE | F | 0.00 | 0.29 | 1.90 | 0.12 | 0.21 | 1.24 |
| | P | 0.9651 | 0.5956 | 0.1750 | 0.7335 | 0.6485 | 0.2708 |
| N | F | 0.49 | 7.03 | 0.02 | 4.01 | 209.91 | 73.71 |
| | P | 0.4876 | 0.0109 | 0.8983 | 0.0510 | 0.0001 | 0.0001 |
| SN | F | 0.43 | 0.60 | 0.62 | 0.00 | 0.59 | 1.48 |
| | P | 0.5152 | 0.4408 | 0.4346 | 0.9734 | 0.4448 | 0.2299 |
| PN | F | 0.19 | 0.28 | 0.02 | 0.02 | 2.42 | 0.61 |
| | P | 0.6617 | 0.5980 | 0.8865 | 0.8764 | 0.1262 | 0.4375 |
| SPN | F | 0.12 | 0.28 | 0.39 | 1.58 | 0.00 | 0.49 |
| | P | 0.7287 | 0.6019 | 0.5377 | 0.2145 | 0.9803 | 0.4894 |
| EN | F | 1.97 | 1.37 | 0.49 | 0.37 | 0.02 | 0.80 |
| | P | 0.1673 | 0.2476 | 0.4893 | 0.5432 | 0.8945 | 0.3759 |
| SEN | F | 1.39 | 0.03 | 0.09 | 1.37 | 0.55 | 0.28 |
| | P | 0.2448 | 0.8714 | 0.7679 | 0.2476 | 0.4609 | 0.6015 |
| PEN | F | 0.12 | 0.00 | 2.51 | 0.48 | 0.10 | 0.01 |
| | P | 0.7353 | 0.9718 | 0.1200 | 0.4932 | 0.7480 | 0.9434 |
| SPEN | F | 0.29 | 0.01 | 0.01 | 0.69 | 0.24 | 0.18 |
| | P | 0.5926 | 0.9219 | 0.9356 | 0.4096 | 0.6293 | 0.6725 |

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

Table 3.3: Analysis of variance table (F-statistic; P-probabilities) of CIE color parameters (L*, a*, b*) for replication (within each plant), plant, and treatment contrasts' effect in the normal group of raw and cooked samples

| Source | Statistic | *Raw* | | | *Cooked* | | |
|---|---|---|---|---|---|---|---|
| | | L* | a* | b* | L* | a* | b* |
| Replication in: | | | | | | | |
| Plant 1 | F | 0.39 | 0.48 | 0.13 | 2.72 | 0.02 | 7.44 |
| | P | 0.5946 | 0.5597 | 0.7562 | 0.2409 | 0.8910 | 0.1122 |
| Plant 2 | F | 45.67 | 3.36 | 4.58 | 28.88 | 2.25 | 20.12 |
| | P | 0.0212 | 0.2083 | 0.1656 | 0.0329 | 0.2721 | 0.0463 |
| Plant | F | 0.96 | 10.52 | 5.37 | 14.47 | 0.00 | 0.93 |
| | P | 0.4304 | 0.0833 | 0.1463 | 0.0627 | 0.9589 | 0.4362 |
| Treatments[1] | | | | | | | |
| S | F | 68.21 | 0.54 | 0.05 | 13.41 | 5.49 | 1.58 |
| | P | 0.0001 | 0.4653 | 0.8323 | 0.0006 | 0.0234 | 0.2154 |
| P | F | 7.24 | 3.62 | 1.16 | 1.76 | 3.30 | 0.04 |
| | P | 0.0098 | 0.0632 | 0.2863 | 0.1916 | 0.0758 | 0.8446 |
| SP | F | 6.69 | 3.09 | 3.11 | 2.77 | 2.39 | 5.13 |
| | P | 0.0128 | 0.0853 | 0.0841 | 0.1027 | 0.1291 | 0.0282 |
| E | F | 0.33 | 0.14 | 0.05 | 0.87 | 5.15 | 0.15 |
| | P | 0.5685 | 0.7116 | 0.8162 | 0.3567 | 0.0278 | 0.6986 |
| SE | F | 0.44 | 0.04 | 0.41 | 0.30 | 0.31 | 1.65 |
| | P | 0.5096 | 0.8458 | 0.5256 | 0.5857 | 0.5792 | 0.2057 |
| PE | F | 0.38 | 0.00 | 0.06 | 2.00 | 0.00 | 0.01 |
| | P | 0.5386 | 0.9995 | 0.8046 | 0.1635 | 0.9751 | 0.9310 |
| SPE | F | 0.86 | 1.78 | 0.10 | 0.04 | 3.57 | 0.69 |
| | P | 0.3594 | 0.1890 | 0.7477 | 0.8376 | 0.0652 | 0.4110 |
| N | F | 0.45 | 12.35 | 0.00 | 2.21 | 144.68 | 85.13 |
| | P | 0.5043 | 0.0010 | 0.9490 | 0.1439 | 0.0001 | 0.0001 |
| SN | F | 0.63 | 1.09 | 0.21 | 0.00 | 6.05 | 0.09 |
| | P | 0.4299 | 0.3027 | 0.6511 | 0.9503 | 0.0177 | 0.7706 |
| PN | F | 1.15 | 0.07 | 0.45 | 0.08 | 3.16 | 0.11 |
| | P | 0.2894 | 0.7935 | 0.5038 | 0.7784 | 0.0820 | 0.7463 |
| SPN | F | 0.35 | 0.16 | 0.00 | 0.06 | 7.80 | 0.01 |
| | P | 0.5582 | 0.6893 | 0.9707 | 0.8108 | 0.0075 | 0.9237 |
| EN | F | 0.08 | 0.00 | 1.45 | 0.23 | 0.57 | 0.36 |
| | P | 0.7725 | 0.9477 | 0.2345 | 0.6339 | 0.4559 | 0.5511 |
| SEN | F | 3.22 | 0.36 | 0.36 | 0.44 | 0.01 | 0.07 |
| | P | 0.0794 | 0.5501 | 0.5540 | 0.5083 | 0.0906 | 0.7950 |
| PEN | F | 0.21 | 0.61 | 0.29 | 0.20 | 0.01 | 0.13 |
| | P | 0.6482 | 0.4403 | 0.5929 | 0.6597 | 0.0932 | 0.7155 |
| SPEN | F | 1.43 | 0.00 | 0.65 | 0.00 | 0.98 | 1.04 |
| | P | 0.2381 | 0.9977 | 0.4232 | 0.0985 | 0.3278 | 0.3120 |

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

Table 3.4: Analysis of variance table (F-statistic; P-probabilities) of CIE color parameters (L*, a*, b*) for replication (within each plant), plant, and treatment contrasts' effect in the dark group of raw and cooked samples

| Source | Statistic | *Raw* | | | *Cooked* | | |
|---|---|---|---|---|---|---|---|
| | | L* | a* | b* | L* | a* | b* |
| Replication in: | | | | | | | |
| Plant 1 | F | 0.98 | 0.01 | 0.01 | 0.05 | 0.04 | 0.02 |
| | P | 0.4260 | 0.9998 | 0.9999 | 0.9994 | 0.8560 | 0.9997 |
| Plant 2 | F | 0.24 | 1.17 | 0.02 | 0.41 | 15.46 | 0.32 |
| | P | 0.6747 | 0.3921 | 0.9954 | 0.5878 | 0.0509 | 0.6266 |
| Plant | F | 4.24 | 0.28 | 17.40 | 16.10 | 0.32 | 2.55 |
| | P | 0.1757 | 0.6509 | 0.0599 | 0.0565 | 0.6309 | 0.2514 |
| Treatments[1] | | | | | | | |
| S | F | 9.94 | 10.05 | 10.26 | 16.33 | 17.25 | 0.31 |
| | P | 0.0028 | 0.0027 | 0.0024 | 0.0002 | 0.0001 | 0.5815 |
| P | F | 0.12 | 0.91 | 8.26 | 1.19 | 1.32 | 20.82 |
| | P | 0.7298 | 0.3441 | 0.0061 | 0.2810 | 0.2567 | 0.0001 |
| SP | F | 10.37 | 1.60 | 1.69 | 0.01 | 2.17 | 6.03 |
| | P | 0.0023 | 0.2119 | 0.2001 | 0.9318 | 0.1473 | 0.0178 |
| E | F | 6.53 | 3.23 | 0.26 | 1.60 | 0.06 | 0.00 |
| | P | 0.0139 | 0.0785 | 0.6110 | 0.2117 | 0.8038 | 0.9965 |
| SE | F | 2.60 | 0.16 | 2.19 | 2.76 | 8.90 | 0.09 |
| | P | 0.1135 | 0.6903 | 0.1452 | 0.1032 | 0.0045 | 0.7614 |
| PE | F | 1.09 | 0.34 | 1.19 | 1.65 | 0.25 | 6.56 |
| | P | 0.3027 | 0.5603 | 0.2817 | 0.2059 | 0.6184 | 0.0033 |
| SPE | F | 0.00 | 1.85 | 0.86 | 0.00 | 0.01 | 0.81 |
| | P | 0.9593 | 0.1799 | 0.3588 | 0.9790 | 0.9309 | 0.3724 |
| N | F | 0.36 | 0.15 | 2.72 | 1.43 | 102.75 | 126.63 |
| | P | 0.5519 | 0.7032 | 0.1059 | 0.2375 | 0.0001 | 0.0001 |
| SN | F | 0.09 | 0.96 | 0.06 | 0.39 | 1.35 | 9.31 |
| | P | 0.7689 | 0.3331 | 0.8124 | 0.5346 | 0.2504 | 0.0037 |
| PN | F | 5.61 | 0.49 | 2.93 | 0.03 | 0.30 | 0.28 |
| | P | 0.0221 | 0.4862 | 0.0936 | 0.8680 | 0.5890 | 0.5967 |
| SPN | F | 1.35 | 0.32 | 6.11 | 0.00 | 11.21 | 0.82 |
| | P | 0.2504 | 0.5740 | 0.0171 | 0.9895 | 0.0016 | 0.3701 |
| EN | F | 2.87 | 0.28 | 2.34 | 3.64 | 0.68 | 3.64 |
| | P | 0.0967 | 0.1011 | 0.1325 | 0.0625 | 0.4125 | 0.0625 |
| SEN | F | 0.97 | 0.73 | 1.51 | 0.18 | 0.48 | 4.92 |
| | P | 0.3293 | 0.3965 | 0.2252 | 0.6713 | 0.4904 | 0.0314 |
| PEN | F | 0.03 | 1.56 | 17.25 | 0.01 | 1.65 | 0.28 |
| | P | 0.8638 | 0.2182 | 0.0001 | 0.9096 | 0.2055 | 0.5998 |
| SPEN | F | 0.05 | 0.21 | 0.01 | 2.47 | 0.00 | 4.73 |
| | P | 0.8328 | 0.6517 | 0.9280 | 0.1226 | 0.9892 | 0.0346 |

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

influenced by salt and phosphate in the injection solutions. Whereas sodium chloride decreased the b* value from 17.63 (control) to 15.47 (salt treated), it increased the b* value to 20.04 for phosphate treated samples.

The three preselected muscle color groups responded differently to the injection treatments. The light group showed a decrease only in the L* value. In contrast, the normal and dark groups showed both increase and decrease of L* and a* values depending on treatment combinations. An increased a* value of raw muscles was observed in all color groups, especially when sodium tripolyphosphate was present in the injection solution. A similar tendency toward decreased lightness (L*) and increased a* value was reported by Young and Chen (1993). In contrast, Allen and co-workers (1998) observed higher L* and lower a* in the marinated breast muscles both from light and dark groups.

**Color of Cooked Samples.** Based on the pink threshold value of a* = 3.8, simulation of the pink defect was achieved in eight of the sixteen treatment combinations in the light group, nine in the normal group, and ten in the dark group (Tables 3.5, 3.6, and 3.7).

Pinking in those samples was subjectively confirmed through visual evaluation in the light booth. The pinking in the eight cooked samples from the light group was directly related to the treatments containing sodium nitrite. The presence of sodium nitrite alone or in combination with other ingredients significantly increased a* value of the cooked fillets in the light group (Table 3.5). Samples containing nitrite also showed lower hue angle values (66.0° – 70.8°) when compared with other treatment combinations (79.2° – 81.7°). The lower the hue angle the redder the samples. The effect was probably caused by both increased a* and decreased b* values. Pink samples in the light group did not show significant change in lightness compared to the rest of the treatments. Chroma values for these samples ranged from 10.5 to 13.2. These data are in agreement with previous published reports on

Table 3.5: Means (±SEM) and contrast estimates for CIE L*, a*, and b* color parameters of cooked breast fillets from light group

| Treatment Combinations[1] | L* | | a* | | b* | |
|---|---|---|---|---|---|---|
| | Mean | Estimate | Mean | Estimate | Mean | Estimate |
| Control | 83.59 ± 0.89 | . . . | 2.60 ± 0.40$^{b}$ | . . . | 14.81 ± 0.76 | . . . |
| S | 83.64 ± 1.15 | -0.2595 | 2.42 ± 0.34$^{b}$ | 0.0030 | 13.24 ± 0.61 | -0.2524 |
| P | 83.92 ± 0.52 | -0.0723 | 2.38 ± 0.29$^{b}$ | -0.0147 | 13.76 ± 1.02 | -0.1330 |
| SP | 82.69 ± 0.62 | -0.6109 | 2.24 ± 0.52$^{b}$ | 0.0739 | 13.96 ± 0.79 | 0.1960 |
| E | 83.39 ± 0.78 | -0.0534 | 2.73 ± 0.25$^{b}$ | -0.0050 | 14.98 ± 0.42 | 0.0292 |
| SE | 82.85 ± 0.42 | -0.0331 | 2.43 ± 0.34$^{b}$ | -0.0179 | 14.12 ± 0.51 | -0.0379 |
| PE | 84.19 ± 0.64 | -0.0793 | 2.11 ± 0.45$^{b}$ | -0.0154 | 14.24 ± 0.57 | -0.0579 |
| SPE | 81.72 ± 1.14 | 0.1150 | 2.36 ± 0.10$^{b}$ | 0.0634 | 14.17 ± 0.70 | -0.3953 |
| N | 82.21 ± 0.43 | -0.1681 | 4.31 ± 0.21$^{a}$ | 0.5008 | 12.48 ± 0.36 | -0.7614 |
| SN | 82.90 ± 0.27 | 0.0056 | 4.41 ± 0.07$^{a}$ | 0.0532 | 10.61 ± 0.80 | -0.2157 |
| PN | 84.30 ± 0.49 | -0.0262 | 4.30 ± 0.16$^{a}$ | 0.1076 | 11.12 ± 1.27 | -0.1389 |
| SPN | 80.91 ± 0.63 | -0.4225 | 4.71 ± 0.23$^{a}$ | 0.0034 | 10.63 ± 0.70 | -0.2471 |
| EN | 82.66 ± 0.53 | 0.1028 | 4.39 ± 0.15$^{a}$ | -0.0092 | 12.14 ± 0.79 | -0.1585 |
| SEN | 83.21 ± 0.53 | 0.3931 | 4.22 ± 0.11$^{a}$ | -0.1028 | 10.84 ± 0.49 | -0.1865 |
| PEN | 83.18 ± 0.46 | -0.2318 | 4.41 ± 0.11$^{a}$ | 0.0446 | 11.59 ± 0.47 | -0.0253 |
| SPEN | 81.23 ± 0.23 | 0.5587 | 4.54 ± 0.22$^{a}$ | -0.1343 | 9.48 ± 0.35 | -0.3018 |

n = 4

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

$^{a-b}$Means within a column with no common superscript differ significantly ($P < 0.05$)

· · ·=Not applicable

Table 3.6: Means (±SEM) and contrast estimates for CIE L*, a*, and b* color parameters of cooked breast fillets from normal group

| Treatment Combinations[1] | L* | | a* | | b* | |
|---|---|---|---|---|---|---|
| | Mean | Estimate | Mean | Estimate | Mean | Estimate |
| Control | 82.58 ± 0.65 | ... | 2.21 ± 0.45$^{c}$ | ... | 15.07 ± 0.66 | ... |
| S | 81.76 ± 0.23 | -0.3789 | 2.59 ± 0.18$^{bc}$ | 0.0757 | 14.44 ± 0.48 | -0.0856 |
| P | 83.27 ± 0.41 | -0.1371 | 2.49 ± 0.18$^{c}$ | 0.0586 | 14.76 ± 0.32 | -0.0134 |
| SP | 81.02 ± 0.79 | -0.3445 | 3.25 ± 0.36$^{b}$ | 0.0998 | 14.31 ± 0.32 | 0.3087 |
| E | 82.28 ± 0.83 | -0.0963 | 2.92 ± 0.32$^{bc}$ | 0.0733 | 15.49 ± 0.37 | 0.0265 |
| SE | 81.70 ± 0.96 | -0.1135 | 2.67 ± 0.19$^{bc}$ | 0.0360 | 14.41 ± 0.41 | 0.1750 |
| PE | 82.31 ± 0.95 | -0.2929 | 2.45 ± 0.37$^{c}$ | 0.0020 | 14.32 ± 0.30 | -0.0118 |
| SPE | 80.00 ± 0.71 | -0.0853 | 4.06 ± 0.09$^{a}$ | 0.2440 | 15.44 ± 0.43 | 0.2262 |
| N | 81.56 ± 0.97 | -0.1538 | 4.20 ± 0.14$^{a}$ | 0.3886 | 12.98 ± 0.61 | -0.6290 |
| SN | 81.02 ± 1.03 | -0.0129 | 4.38 ± 0.12$^{a}$ | -0.1589 | 11.44 ± 0.66 | -0.0400 |
| PN | 82.16 ± 0.91 | -0.0585 | 4.47 ± 0.27$^{a}$ | -0.1148 | 12.44 ± 0.89 | 0.0443 |
| SPN | 80.63 ± 0.44 | 0.0996 | 4.09 ± 0.25$^{a}$ | -0.3609 | 12.30 ± 0.34 | 0.0262 |
| EN | 82.55 ± 1.29 | 0.0992 | 4.41 ± 0.32$^{a}$ | -0.0485 | 12.57 ± 0.69 | -0.0818 |
| SEN | 81.19 ± 0.79 | -0.2759 | 4.53 ± 0.20$^{a}$ | 0.0153 | 11.98 ± 1.08 | -0.0712 |
| PEN | 81.80 ± 0.94 | -0.1834 | 4.48 ± 0.25$^{a}$ | 0.0109 | 11.89 ± 0.66 | -0.1000 |
| SPEN | 79.07 ± 0.55 | -0.0156 | 4.51 ± 0.23$^{a}$ | -0.2556 | 12.48 ± 0.37 | -0.5575 |

n = 4

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

$^{a-c}$Means within a column with no common superscript differ significantly ($P < 0.05$)

$\cdots$=Not applicable

Table 3.7: Means (±SEM) and contrast estimates for CIE L*, a*, and b* color parameters of cooked breast fillets from dark group

| Treatment Combinations[1] | L* | | a* | | b* | |
|---|---|---|---|---|---|---|
| | Mean | Estimate | Mean | Estimate | Mean | Estimate |
| Control | 82.26 ± 0.57 | ... | 3.24 ± 0.68$^{e}$ | ... | 15.78 ± 0.85 | ... |
| S | 79.46 ± 0.77 | -0.3815 | 2.96 ± 0.23$^{ef}$ | 0.1675 | 15.67 ± 0.51 | 0.0389 |
| P | 80.81 ± 0.67 | -0.1029 | 3.03 ± 0.32$^{ef}$ | 0.0463 | 15.08 ± 0.18 | -0.3198 |
| SP | 79.16 ± 0.54 | -0.0162 | 4.29 ± 0.10$^{bcd}$ | 0.1189 | 16.14 ± 0.36 | 0.3443 |
| E | 80.91 ± 0.93 | -0.1195 | 3.08 ± 0.31$^{ef}$ | 0.0100 | 16.09 ± 0.44 | 0.0003 |
| SE | 80.24 ± 0.20 | 0.3137 | 3.51 ± 0.12$^{de}$ | 0.2407 | 16.33 ± 0.25 | -0.0428 |
| PE | 81.72 ± 0.43 | 0.2421 | 2.27 ± 0.40$^{f}$ | -0.0404 | 12.64 ± 1.08 | -0.4334 |
| SPE | 79.79 ± 1.29 | -0.0100 | 4.29 ± 0.17$^{bcd}$ | 0.0140 | 15.49 ± 1.03 | -0.2525 |
| N | 81.88 ± 0.86 | -0.1129 | 4.79 ± 0.16$^{ab}$ | 0.4089 | 15.78 ± 0.61 | -0.7887 |
| SN | 80.42 ± 0.33 | 0.1181 | 5.00 ± 0.36$^{ab}$ | -0.0939 | 11.69 ± 0.46 | -0.4278 |
| PN | 81.58 ± 0.68 | -0.0315 | 5.08 ± 0.39$^{ab}$ | -0.0439 | 10.97 ± 0.13 | -0.0746 |
| SPN | 78.88 ± 0.14 | -0.0050 | 4.65 ± 0.50$^{c}$ | -0.5403 | 12.28 ± 0.15 | -0.2537 |
| EN | 80.03 ± 0.58 | -0.3603 | 4.26 ± 0.22$^{bcd}$ | 0.0667 | 14.08 ± 0.24 | 0.2675 |
| SEN | 78.99 ± 1.50 | 0.1612 | 5.63 ± 0.15$^{a}$ | 0.1121 | 13.10 ± 0.72 | -0.6218 |
| PEN | 79.45 ± 0.21 | -0.0431 | 4.77 ± 0.31$^{ab}$ | 0.2071 | 12.36 ± 0.25 | 0.1481 |
| SPEN | 79.50 ± 0.73 | 1.1875 | 5.56 ± 0.25$^{a}$ | 0.0043 | 10.53 ± 0.24 | -1.2200 |

n = 4

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

$^{a-f}$Means within a column with no common superscript differ significantly ($P < 0.05$)

$\cdots$=Not applicable

the significant role played by sodium nitrite manifesting pinking for concentration as little as 1 ppm (Ahn and Maurer, 1989a; Heaton et al., 2000). The pinking in the light group was not affected by a combination of salt, phosphate, and erythorbate; these ingredients, in combination are known to influence pH, reducing conditions, and the solubility of proteins. However, since the light group has well established low initial pH (Fletcher, 1999), it is unlikely that salt, phosphate, and erythorbate would cause enough changes in the in situ conditions to promote pinking.

A significant effect for sodium nitrite was found for the remaining color groups as well (Tables 3.3 and 3.4). The positive effect (increased a* value) was indicated by higher positive contrast estimates for the light, normal, and dark groups, respectively (Tables 3.6 and 3.7). Estimated chroma and hue angle in the normal and dark groups showed the same trends as in the light group. Chroma values in pink samples containing sodium nitrite in the normal and dark groups ranged from 12.2 to 13.6, and from 11.9 to 14.7, respectively. Hue angles were affected both by significantly higher a* and by significantly lower b* values: 68.76° – 71.5° in the normal group, and 62.1° – 69.5° in the dark group. It was also found that in all three color groups the presence of sodium nitrite significantly decreased b* value of the cooked fillets (Tables 3.2, 3.3, 3.4, 3.5, 3.6, and 3.7). A similar significant decrease in b* values caused by sodium nitrite was reported by Ahn and Maurer (1989a). In addition to the eight treatments which induced pinking, a combination of sodium chloride, tripolyphosphate, and erythorbate also modified in situ conditions to an extent that produced pinking of cooked samples from the normal and dark groups. Those a* values exceeded the subjective pink threshold. The values were significantly different from a* values of the fillets with no pink defect (Tables 3.6 and 3.7). Through a significant effect on the a* value only a combination of sodium chloride with tripolyphosphate produced pinking in cooked samples from the dark group (Table 3.7). Estimated chroma values for pink samples from the normal and

dark groups ranged from 15.9 to 16.7. These chroma values for the normal and dark groups were similar to those of their respective control samples (15.2 – 16.1). Pink samples from the normal and dark groups lacking sodium nitrite had b* and chroma values similar to those of the control samples, showing that a* value was the main cause for lower hue angle and pinking in those samples. The hue angles of pink samples injected with sodium chloride and tripolyphosphate or the combination of sodium chloride, tripolyphosphate, and erythorbate ranged from 74.4° to 75.2°, whereas the control showed a range of 78.6° to 81.7°. Janky and Froning (1972) have previously shown that sodium erythorbate with polyphosphate increases redness of metmyoglobin in a meat system. The significant role of added phosphates, alone or in combination with sodium chloride, in increasing a* values has also been confirmed previously (Ahn and Maurer, 1989a,b).

**Reflectance Spectra.** The spectra for raw samples (Figure 3.1-A) in all color groups were typical of myoglobin or mixtures of myoglobin (reflectance minimum ∼560 nm) and metmyoglobin (reflectance minimum ∼630 nm). However, in samples injected with a combination of salt, tripolyphosphate with erythorbate there was some evidence of oxymyoglobin formation as indicated by the reflectance minima at 540 nm and 580 nm (Figure 3.1-B). Millar and others (1993) also reported some evidence of oxymyoglobin in poultry meat. For the light, normal, and dark groups (Figures 3.1-C,D), characteristic spectral curves of nitrosomyoglobin were found in raw samples injected with sodium nitrite or its combinations. These spectra had reflectance minima at about 421 nm, 548 nm, and 579 nm. Similar spectral curves of nitrosomyoglobin were found by Millar and others (1996), even at low concentrations. The nitric oxide myoglobin that appears red in raw meat may be formed in the raw meat without the intermediate step of nitric oxide metmyoglobin formation (Livingston and Brown, 1981). When sodium nitrite was combined with sodium

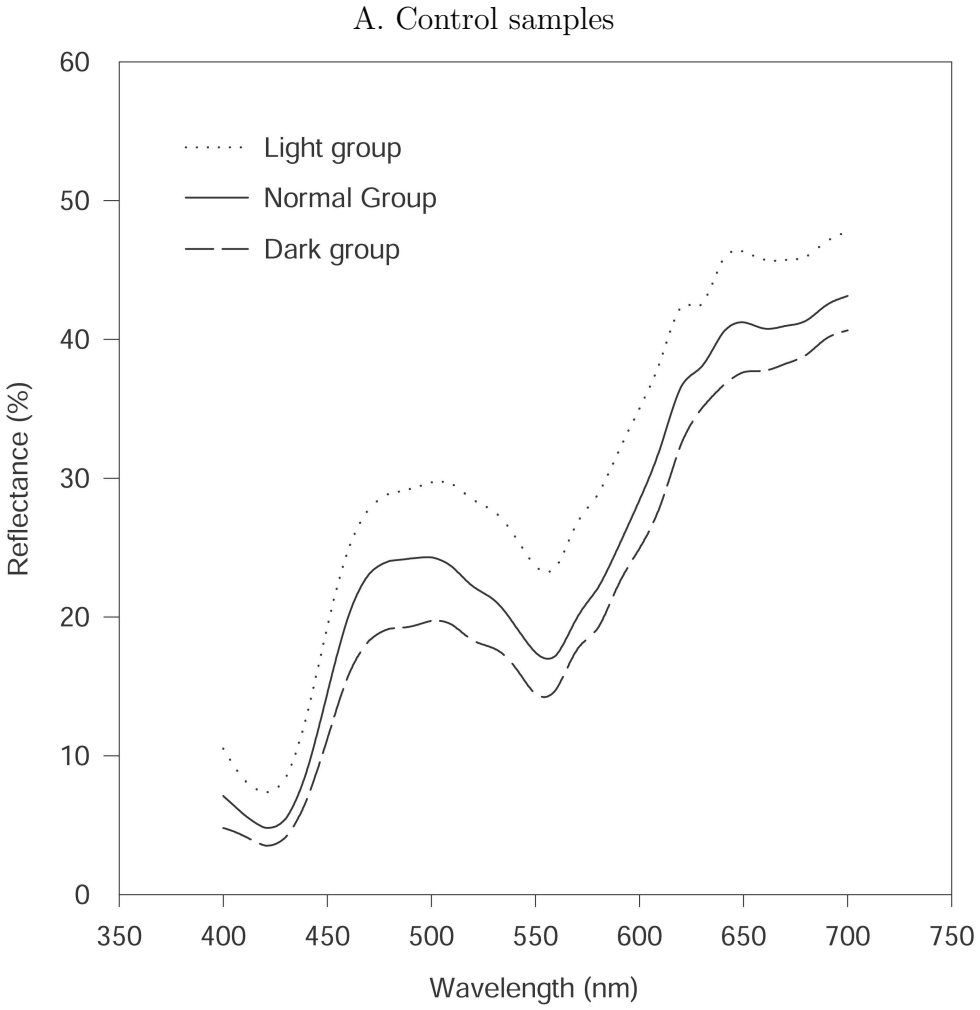


Figure 3.1: Reflectance spectra of raw fillets from light, normal, and dark groups

B. Samples injected with SPE and SP[1]

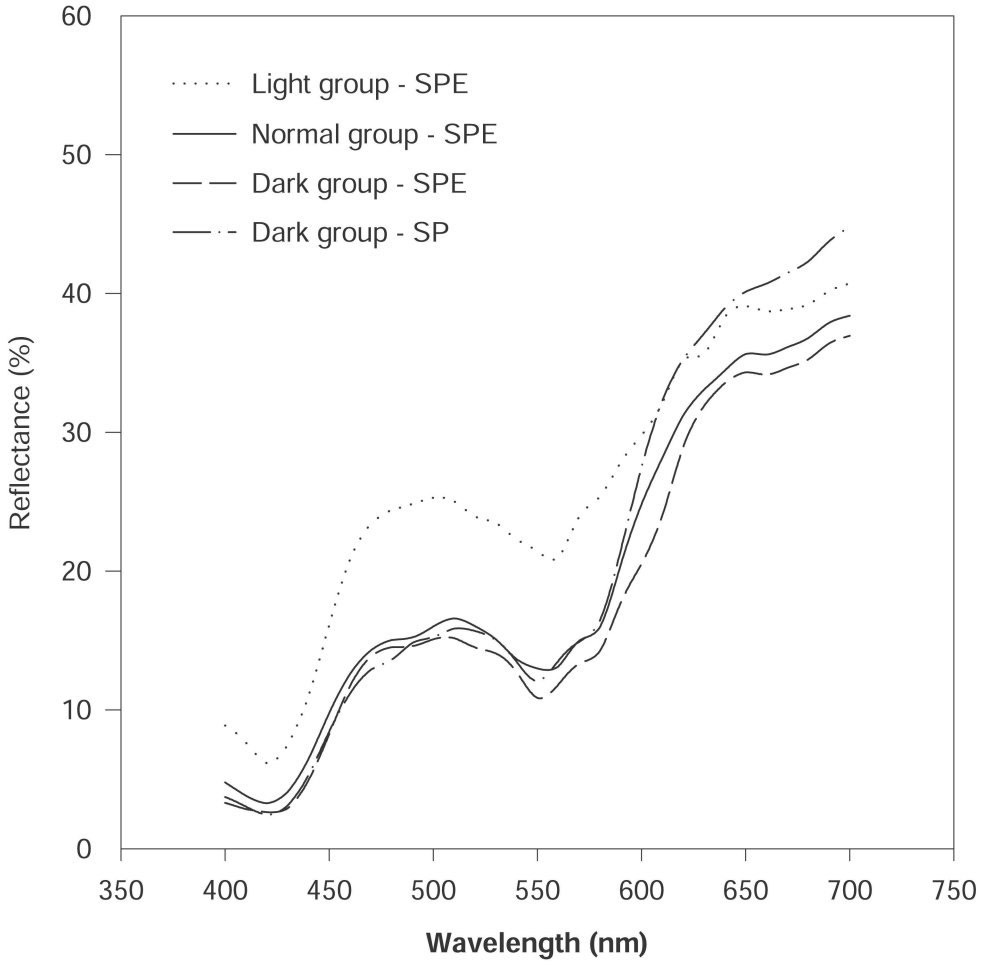


Figure 3.1: Cont.

[1]SPE - injected with a combination of sodium chloride, sodium tripolyphosphate, and sodium erythorbate; SP - injected with sodium chloride and sodium tripolyphosphate

C. Samples injected with SN[1]

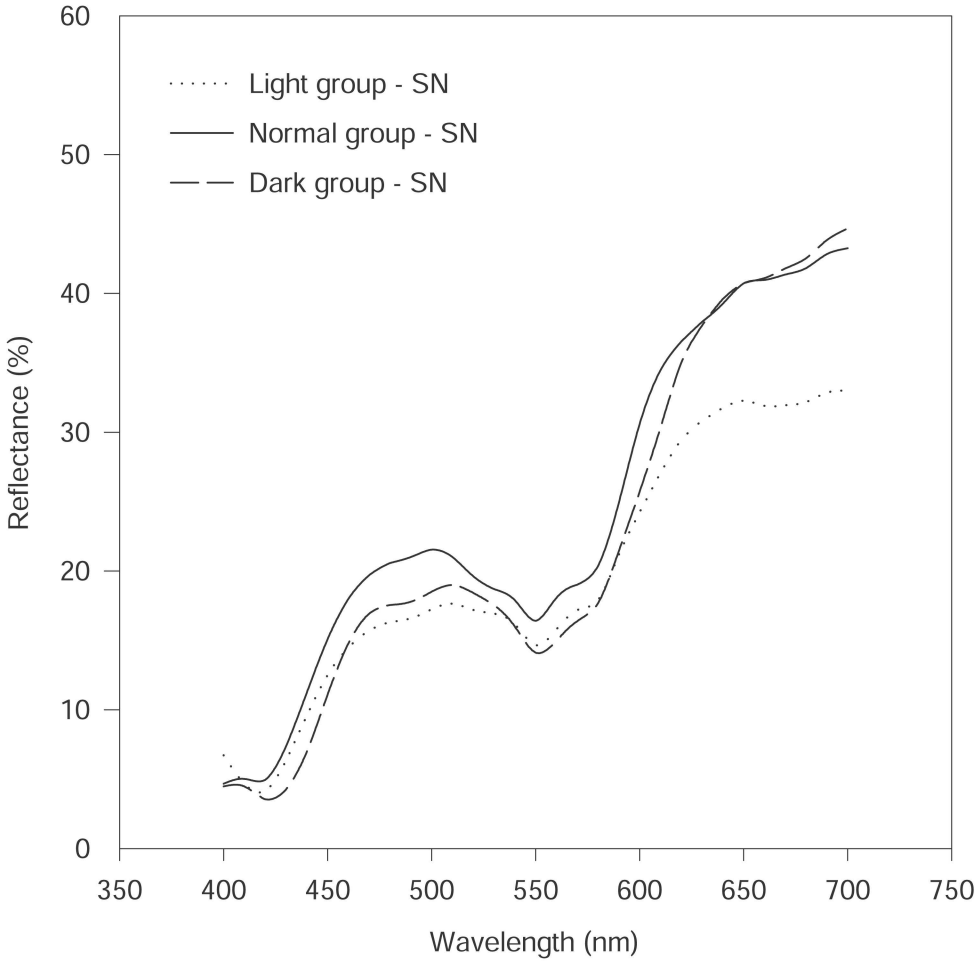


Figure 3.1: Cont.

[1]SN - injected with sodium chloride and sodium nitrite

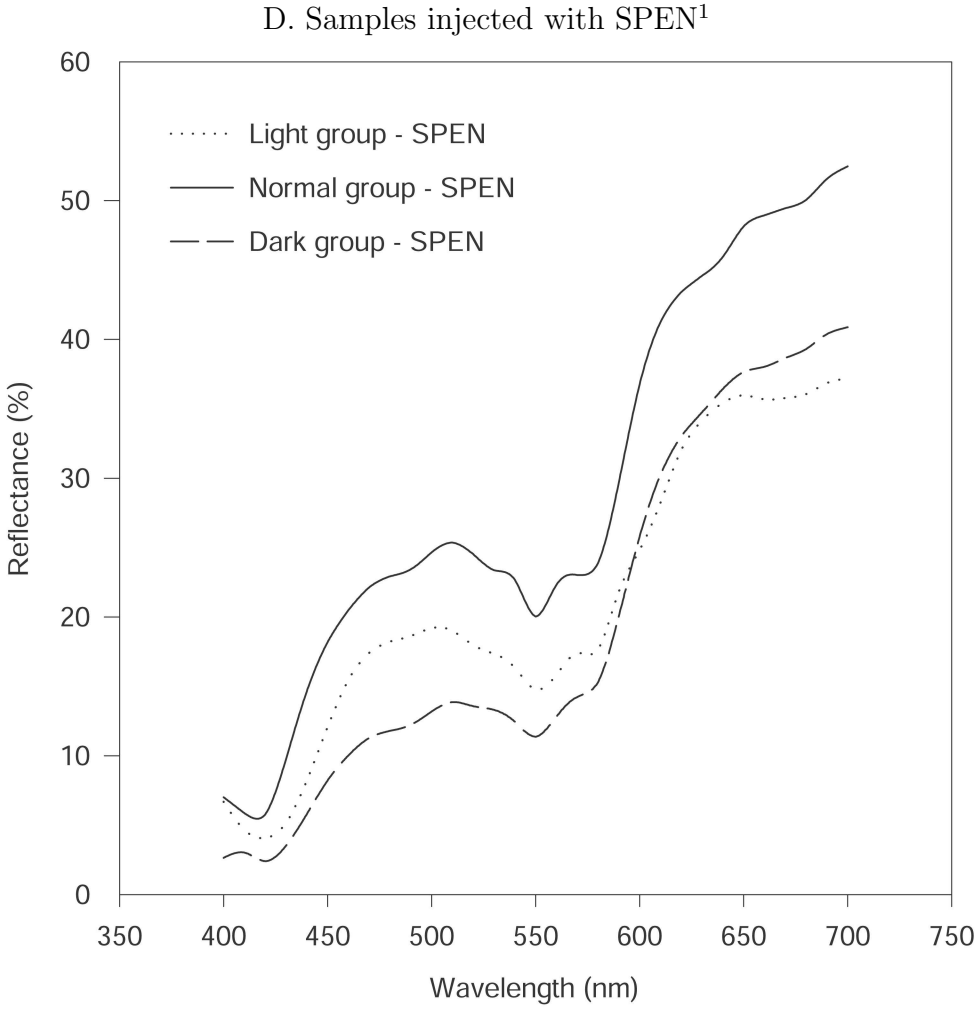


Figure 3.1: Cont.

[1]SPEN - injected with a combination of sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite

chloride, tripolyphosphate, and erythorbate, higher reflectance values occurred for all three groups than when one of the three compounds was missing (Figure 3.1-C,D).

The fact that no reflectance minima were identified at about 542 nm and 580 nm in the cooked control samples in all groups, indicated that denaturation of myoglobin had occurred (Figure 3.2-A). The very pronounced minimum at 550 nm was found for normal and dark control cooked fillets may be an indication of the presence of pink ferrocytochrome c (Ahn and Maurer, 1989b). However, those samples exhibited no pinking after cooking. The reflectance spectra from normal and dark groups injected with a combination of salt, phosphate, and erythorbate (pink samples) showed that pinking was mainly due to reduced hemochromes (Figure 3.2-B). These spectral curves, with reflectance minima at about 555 nm, 520 – 530 nm, and 415 – 420 nm, are exactly those to be expected for reduced hemochromes (Brown and Tappel, 1957; Tappel, 1957; Akoyunoglou et al., 1963; Ledward 1974; Girard et al., 1990; Ahn and Maurer, 1990a; Cornforth et al., 1986; Ghorpade and Cornforth, 1993). Pink ferrohemochromes usually have reflectance minima very similar to brown ferrihemochromes. Ferrohemochromes that are formed by reduction of ferrihemochromes cause a shift in Soret, alpha, and beta minima toward the longer wavelength (Akoyunoglou et al., 1963). There is a problem in identifying the nitrogenous compound as a sixth ligand coordinating to the pigment molecule. It is difficult to distinguish between different hemochromes by reflectance measurement. They may be characteristic of many different compounds (Cornforth et al., 1986; Ghorpade and Cornforth, 1993). In addition, an absorption maxima (reflectance minima) at 550 nm, 520 nm, and 415 nm, characteristic of ferrocytochrome c, have been reported by Ahn and Maurer (1990b) and Girard and others (1990). Diffused reflectance minima at 540 nm and 565 nm, characteristic of nitrosohemochromes (Brown and Tappel, 1957), were found in all spectral curves in cooked samples containing sodium nitrite. Examples of these spectra are presented in Figures 3.2-C,D. The intensities of these minima were more

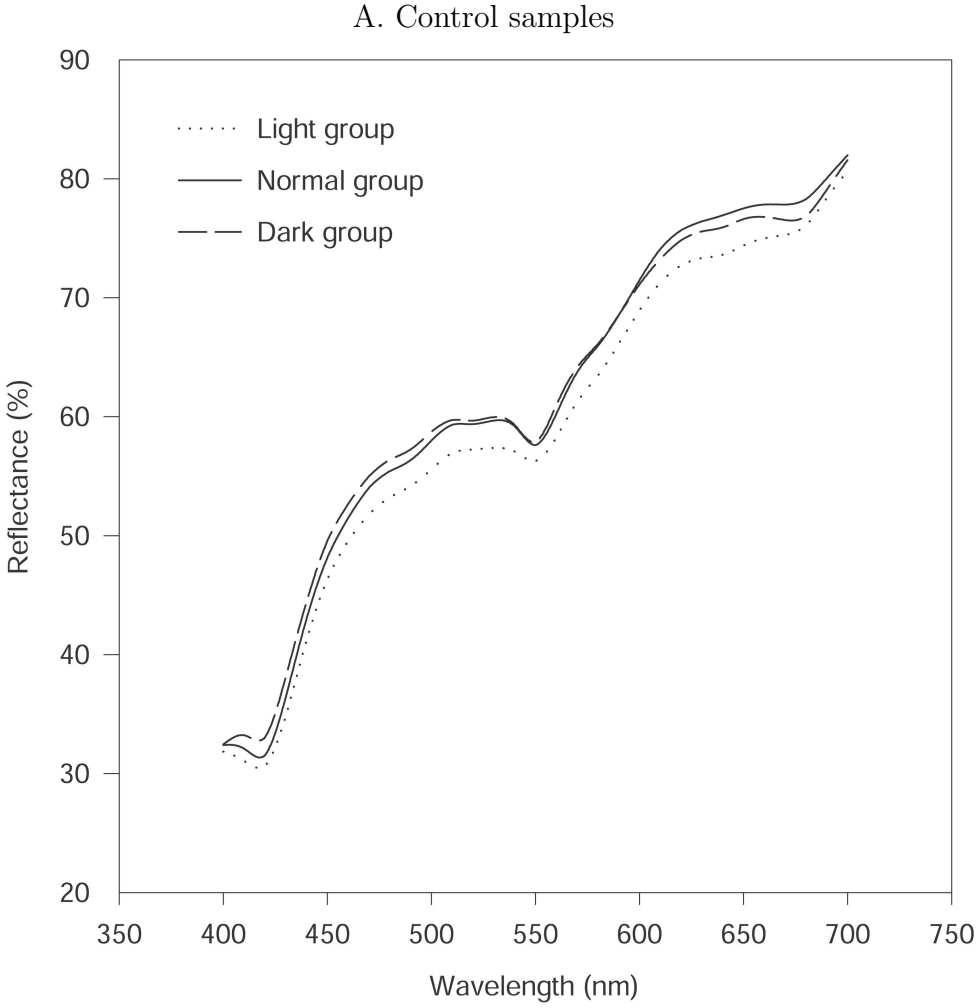


Figure 3.2: Reflectance spectra of cooked fillets from light, normal, and dark groups

B. Samples injected with SPE and SP[1]

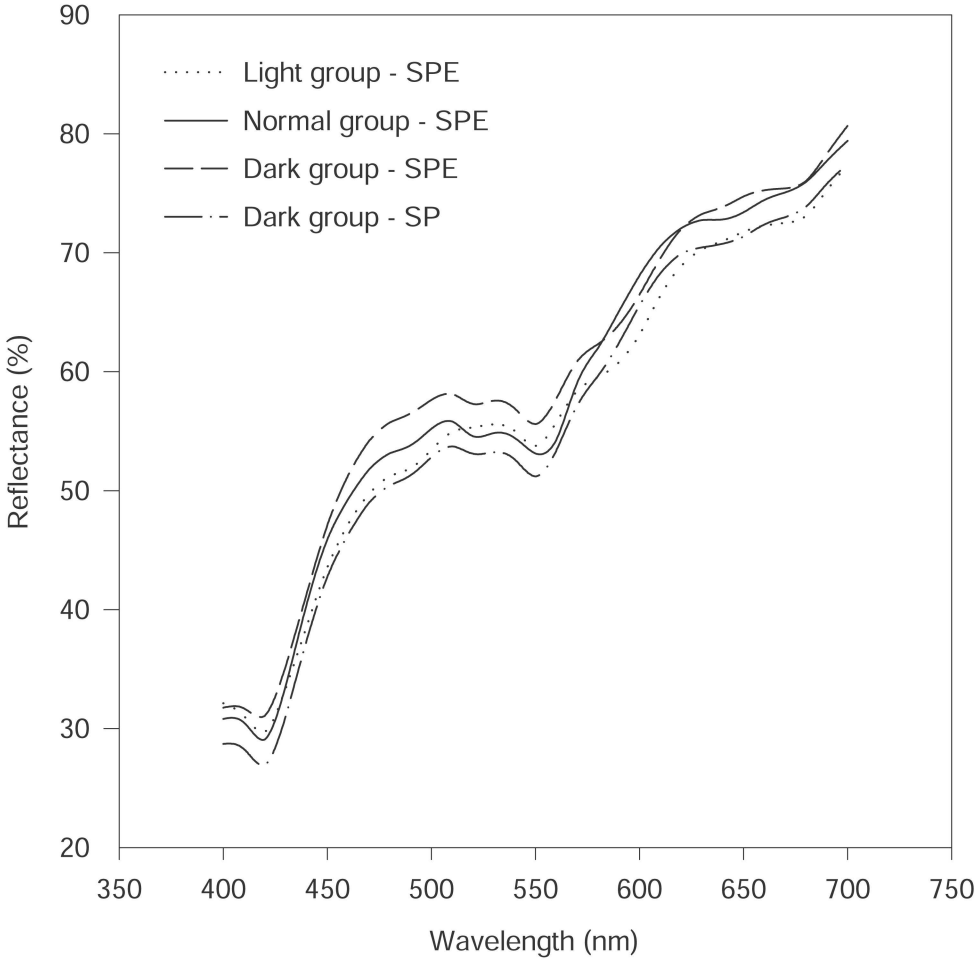


Figure 3.2: Cont.

[1]SPE - injected with a combination of sodium chloride, sodium tripolyphosphate, and sodium erythorbate; SP - injected with sodium chloride and sodium tripolyphosphate

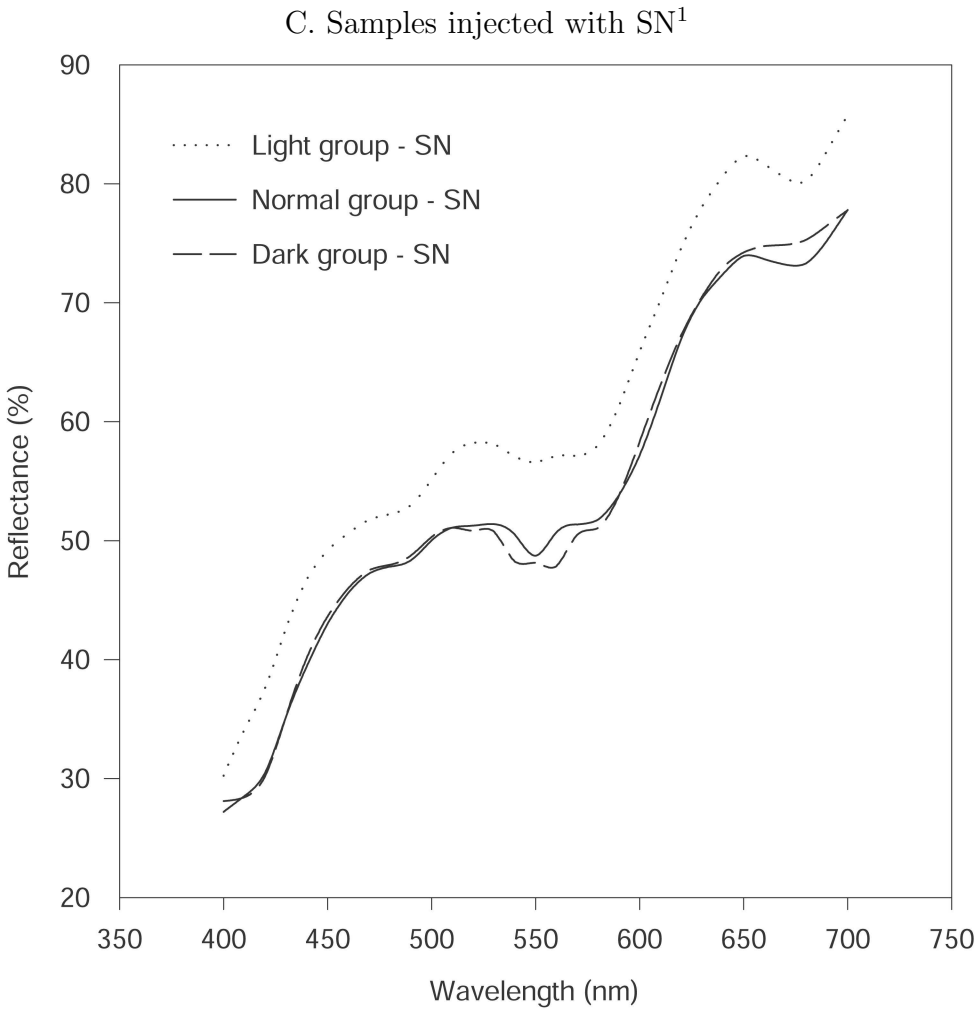


Figure 3.2: Cont.

[1]SN - injected with sodium chloride and sodium nitrite

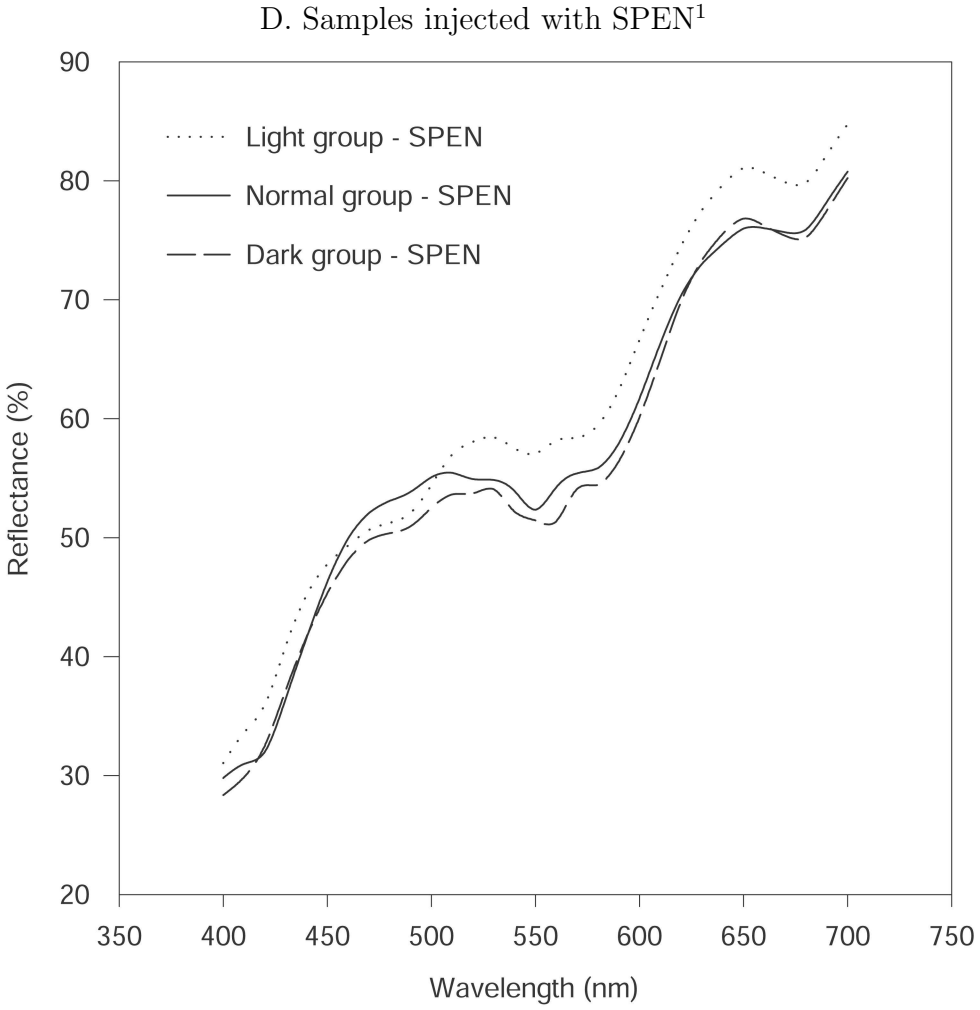


Figure 3.2: Cont.

[1]SPEN - injected with a combination of sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite

pronounced in cooked fillets from the normal and dark groups than from the light group. Even for samples from the normal and dark groups which were injected with sodium nitrite in combination with other ingredients, minima between 520 and 530 nm were present (Figures 3.2-C,D). This minima range is typical of non-nitrosyl hemochromes (Ahn and Maurer, 1990; Ghorpade and Cornforth, 1993).

Reflectance spectra measurement of meat surfaces cannot provide accurate information on pigment concentration because of variation in the light scattering properties of meat; spectra can provide information on the state of pigment oxygenation or oxidation and on the presence of compounds that are not extractable (hemi- and hemochromes). Reflectance measurements were obtained to seek information whether the presence of the simulated pink defect in cooked samples is related to the presence of certain pigment classes.

## 3.5 Conclusions

It is possible to simulate a pink defect in cooked chicken breast fillets using the in situ conditions of raw meat that were induced by sodium chloride, sodium tripolyphosphate, and sodium erythorbate. The presence of sodium nitrite had the most significant effect on pinking, even at very low levels such as 1 ppm. The results proved the reproducibility of the pink defect because no significant ($P > 0.05$) plant-effect was found. The ingredients used to induce in situ conditions have evidently modified the susceptibility of pigments to denaturation, pH , reducing conditions, and ligands reactivity with the heme iron. The relationship of the induced in situ conditions and simulated pinking is the subject of a subsequent study.

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# Chapter 4

## Relating Induced In Situ Conditions of Raw Chicken Breast Meat to Pinking[1]

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## 4.1 Abstract

The objective was to simulate the pink color defect in cooked chicken breast meat with treatment combinations that would induce measurable changes in the conditions of raw meat. In addition, the feasibility of using induced raw meat conditions to develop a logistic regression model for prediction of pinking was studied. Approximately 960 breast fillets from two plants with two replications were used for inducing in situ conditions with sixteen combinations of sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite (present and not present). Muscles in all treatments were subjected to individual injections, followed by tumbling, cooking and chilling. Raw samples were analyzed for pH, oxidation-reduction potential, and pigments. Results indicated a significant role of induced in situ conditions of raw meat in the occurrence of pinking. Presence of 1 ppm or more of sodium nitrite in raw meat produced significant pinking of cooked meat. The light muscle color group was the least affected and the dark group was the most affected by induced pH, oxidation-reduction potential conditions, metmyoglobin, and nitrosopigment content. The predictive ability of the logistic model was more than 90% with nitrosopigment, pH, and reducing conditions to be the most important factors. Moreover, the validation of the model was confirmed by close association between observed pink samples and those predicted as pink.

(*Keywords:* pinking, raw chicken meat, prediction, logistic model)

## 4.2 Introduction

The pink color defect in white poultry meat has been a subject of research for over forty years. When and why the pinking phenomenon appears is unclear. Therefore, it is challenging to study the pinking problem as it happens. Currently, major attention focuses on processing factors such as marinating ingredients, processing water, endpoint cooking temperature, and cooking methods. Froning and others (1968) stated that pinking occurs in cooked turkey meat after refrigeration; Girard and others (1989) found that pinking remained in turkey muscle after cooking to 85°C. Since then a number of research papers has been presented that investigated the cooked meat conditions related to pinking. The presence of nitrites in a concentration of 1 ppm or more causes pinking in cooked white poultry meat (Froning et al., 1969; Nash et al., 1985; Ahn and Maurer, 1987, 1989a; Heaton et al., 2000). Several studies have shown that different processing and marinating ingredients change pH and oxidation-reduction potential of cooked meat significantly enough to induce pinking (Cornforth et al., 1986; Ahn and Maurer, 1989a,b; Trout 1989). A high pH in cooked meat (>6.4) is favorable for the heme-complex-forming reactions of pigments with most ligands (Ahn and Maurer, 1990). High pH also decreases the susceptibility of myoglobin to heat denaturation, allowing greater reactivity of pigments (Trout, 1989; Girard et al., 1990; Young et al., 1996a).

Several researchers attempted to reduce pinking using different ingredients, such as ligands binding to heme iron, without formation of pink color in cooked products (Schwarz et al., 1997, 1999; Slesinski et al., 2000a,b). However, the mechanism of pink color reduction is not yet established. Moreover, adding the non-pink generating ligands may or may not be effective depending on how the raw meat endogenous conditions were altered by processing procedures. There is not much information available relating raw meat conditions to pinking. There is a need for development

of control methods and processing procedures to eliminate the development of the pink quality defect. In our lab we made an attempt to use a simulated pink defect as a research tool to investigate how the occurrence of pinking is effected by certain endogenous conditions of raw meat.

Logistic regression is a predictive statistical analysis, like linear regression, that can simultaneously relate both continuous and discrete independent variables to a binary (dichotomous) response (Afifi and Clark, 1990; Miller et al., 1991; Hand, 1992). The logistic regression model uses the explanatory variables to predict the probability of a certain event's occurrence. Logistic regression does not assume linearity of relationship between the input variables and the dependent (occurrence or nonoccurrence of an event) and does not require normally distributed variables (Hand, 1992). The logistic model is written as

$$Prob\ (event) = \frac{1}{1 + e^{-Z}}$$

where $z = \alpha + \beta' x_i$.

Logistic regression applies a maximum likelihood estimation after transforming the dependent variable into a logit variable (the natural log of the odds of the event occurring or not).

$$logit\ (Prob\ (event)) = log\left(\frac{Prob\ (event)}{1 - Prob\ (event)}\right) = \alpha + \beta' x_i$$

where

$1 - Prob\ (event) = Prob\ (not\ event)$,

$\alpha$ is the intercept parameter,

$\beta$ is the vector of slope parameters, and

$x_i$ is the vector of explanatory variables.

The primary objective was to measure how changes in the in situ conditions (raw meat) caused by sodium chloride, sodium tripolyphosphate, sodium erythorbate, and

sodium nitrite are related to pinking. The secondary objective involved relating the induced in situ conditions of raw meat to the occurrence of pinking in cooked meat using a binary logistic regression model approach with external validation of the predictive model.

## 4.3 Materials and Methods

### Sample Collection and Preparation

The experiment employed boneless, skinless, chicken breast fillets obtained from processing plants, and preselected based on three color groups: lighter than normal ("light"), normal ("normal"), and darker than normal ("dark") (Fletcher, 1999; Fletcher et al., 2000). Fillets were first sorted based on visual appearance at the deboning line or at the beginning of a "further processing" line. Sorting was verified using a Hunter Lab reflectance colorimeter[2]; fillets were selected based on the medial surface (bone side) lightness (L*) values. L* values for the three color groups were: $L^* < 47$ for the dark group, $47 < L^* < 50$ for the normal group, and $L^* > 50$ for the light group. The samples segregated according to color group (light, normal, or dark) were packed with ice and transported to the laboratory.

Approximately 960 breast fillets were collected from two plants. Two hundred forty fillets (eighty per each of the three color groups) were collected per replication during two or three visits to each of the two processing plants.

All breast fillets, 24 h postmortem, were vacuum packed[3] into sampling polyethylene bags. Samples were labelled and coded individually, then frozen and stored at $-18°C$ for up to 3 weeks before the experiment. Experimentation commenced after samples were thawed at 4–5°C (approximately 10–12 h). There were four treatment

[2]MiniScan/XE 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA 22090

[3]Ultravac 250 KOCH Packaging, Division of KOCH Supplies, Inc., Kansas City, MO 64108

factors: sodium chloride[4] (1g/100g meat), sodium tripolyphosphate[5] (0.5g/100g meat), sodium erythorbate[6] (0.0546g/100g meat), and sodium nitrite[7] (1 ppm). Two levels of factors were employed: present and not present. Therefore, sixteen treatment combinations were used. The level of each factor was chosen according to standard regulations and common industry applications. The injection method (12% of meat weight basis) was used to incorporate ingredients into the samples. The ingredients were prepared in stock solutions: 0.1% of sodium nitrite, 10% of sodium tripolyphosphate, and 1% of sodium erythorbate. Next, the stock solutions were combined to obtain the final volume required for each sample so that a 12% injection provided 0.5% sodium tripolyphosphate, 0.0546% sodium erythorbate, and 1 ppm sodium nitrite. For a 200 g fillet, the injection was prepared in a 50 ml beaker as follows: (1) 10 ml of 10% tripolyphosphate was added to the beaker; (2) sodium chloride in the amount of 2 g was added with stirring; (3) 0.2 ml of 0.1% sodium nitrite stock solution was stirred into it; (4) sodium erythorbate (1%) in the amount of 10.92 ml was stirred in as the last ingredient. Finally, distilled water was added to bring the final volume to 24 ml — that is 12% of the weight of the fillet. Separate volumes of injection solutions were prepared and injected. Sixty-four injection solutions were required (4 muscles x 16 treatment combinations). The uniformly-spaced multiple injections were carried out with a 35 ml syringe[8] equipped with a stainless steel 16 hole spray needle[9]. Once injected, samples were packed individually in moisture-impermeable polyethylene bags, then sealed and tumbled[10] for 15 min. Each time, four individually packed muscles (∼25% load) from the same treatment

[4]Catalog No. S271-3, Fisher Scientific, www.fishersci.com
[5]Catalog No. T-5883, Sigma Chemical Co., St. Louis, MO 63178
[6]Catalog No. 49,633-2, Aldrich Chemical Company, Inc., Milwaukee, WI 53233
[7]Catalog No. S-2252, Sigma Chemical Co., St. Louis, MO 63178
[8]Sherwood Medical Company, St. Louis, MO 63103
[9]KOCH No. 30410306, KOCH Supplies, N. Kansas City, MO 64116
[10]Laboratory-made small tabletop tumbler (O.D 40 cm, two equally spaced webs)

were tumbled for 15 min at 45 RPM with 1.0 kg of ice in order to keep the product temperature at about 0°C. Tumbled samples were refrigerated overnight (12 h) at 4–5°C for equilibration before further experimentation.

The following morning, two of four fillets from the experimental unit were designated for raw analysis, and the other two were subjected to cooking. The latter two fillets, placed in aluminum mini broiler pans[11] and covered with aluminum foil, were cooked in a 167°C convection oven[12] to an internal temperature of 74°C. After cooking, samples were first cooled to 48°C within 45 minutes in a temperature controlled laboratory where the temperature was maintained constant at 21°C, and following that the samples were cooled to 4.4°C by placing the samples in the walk-in environmental cooler maintained at 4°C. The total time to achieve the final temperature of the samples was achieved within 4 h. The internal temperature of the product was monitored throughout the cooking and cooling process using K-type chromel-alumel thermocouples inserted in the center of the cranial end of a muscle. An HP BenchLink Data Logger 1.1/data acquisition unit[13] was used to monitor samples temperature during the cooking and cooling process.

**Judgment of Pinking**

The subjective pink threshold established previously at CIE a* = 3.8 (with L* ranging from 79 – 84, and b* from 10 – 16) was used in judging the existence of pinking in the cooked samples (Holownia et al., 2002). The threshold had been produced by injecting samples from the normal group with 1% sodium chloride and sodium nitrite solutions (1, 2, 3, 4, and 5 ppm). The experimental cooked samples

[11]Mini Broiler Pans Size: 20 cm x 16.2 cm x 3.3 cm, HANDI-FOIL Corporation, Wheeling, IL 60090

[12]STABIL-THERM Lindberg/Blue M Electric Oven, GS LINDBERG/BLUE M, Blue Island, IL 60406

[13]HP 34970A Data Acquisition/Switch Unit with 20 Channel HP 34901A Multiplexer, Hewlett-Packard Company, Loveland, CO 80537

were sliced horizontally into lengthwise halves. Immediately, color was objectively evaluated in the light booth (Illuminant D65), and an a* value was measured with a Hunter lab colorimeter. Four color measurements were taken on each half of the fillet; two readings in the anterior and two in the posterior portion of the muscle, rotating the samples 90° between measurements. The average value of eight measurements (four per half) was used. Both visual and instrumental results were used to judge the presence or absence of pinking.

In the following sections the raw meat variables are analyzed and referred to the pinking that was judged after additional samples from the same treatment combinations were cooked. This way, results of raw meat conditions are always discussed in association with the pink defect or pinking that by assumption means the defect in cooked meat.

## Analyses

**pH Measurement.** The muscle pH of raw meat was determined using the iodoacetate method described by Jeaocke (1977). For this analysis, approximately 25 g of meat tissue was removed from anterior portion of the fillet and ground. Duplicate pH measurements were determined for each sample by dispersing 2 g of each sample in 25 ml of 5 mM iodoacetate[14] containing 150 mM KCl[15]. After homogenizing[16] for 30 s at 3000 rpm, the pH of the slurry was measured with a pH meter[17] equipped with a combination pH electrode[18]. Mean values of the duplicate observations were recorded.

[14]Catalog No. I-4386, Sigma Chemical Co., St. Louis, MO 63178
[15]Catalog No. P-4504, Sigma Chemical Co., St. Louis, MO 63178
[16]LabTek Homogenizer, Omni International, Inc., Gainesville, VA 22065
[17]ORION, Model 525A+, Thermo Orion, Beverly, MA 01915
[18]Catalog No. 8172BN, Thermo Orion, Beverly, MA 01915

**Oxidation-Reduction Potential (ORP).** A platinum combination redox electrode[19] was firmly placed in the center of cranial end of a raw breast fillet (3–5°C). ORP readings (mV) were recorded after 5 minutes of stabilization (Moiseev and Cornforth, 1999).

**Percent of Metmyoglobin.** Myoglobin was extracted from the raw meat using an ice-cold (0°C) 0.04 M phosphate buffer[20], pH = 6.8 (Warris, 1979). Twenty five grams of the sample was removed from the anterior portion of the fillet and ground. Next, 5 g was homogenized with 50 ml of buffer at 10,000 rpm for 20 s. The homogenized samples were centrifuged[21] for 30 min at 15,000 g at 4°C. The supernatant was then filtered through a Whatman No.1 filter paper[22]. Metmyoglobin (% of total) was calculated based on the absorbance of clarified extracts at 525, 572, and 700 nm (Krzywicki, 1979) using a HP 8451A Diode Array Spectrophotometer[23]. Metmyoglobin concentration was calculated using the formula:

$$\%\ Metmyoglobin = \left[1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}}\right)\right] 100$$

where, $A_\lambda = Absorbance\ at\ \lambda,\ nm.$

**Nitrosopigment and Total Pigment.** The amounts of nitrosopigment pigment and total pigment in raw samples were measured using Hornsey's method (Hornsey, 1956). Nitrosopigment and total pigment were extracted from 5 g sample using an 22 ml 80% acetone[24] solution. To minimize pigments' fading, neutralized cysteine[25] solution (0.5%) was added to the sample in the amount of 0.5 ml before

[19]Catalog No. 9778BN, Thermo Orion, Beverly, MA 01915

[20]$Na_2HPO_4$Cat No. S-9763 and $NaH_2PO_4.H_2O$ Catalog No. S-9638, Sigma Chemical Co., St. Louis, MO 63178

[21]Beckman Model J2-21M, Rotor JA-14, Beckman Instruments, Inc., Palo Alto, CA 94304

[22]Catalog No. 1001 125, Whatman Inc., Clifton, NJ 07014

[23]Agilent Technologies (Hewlett-Packard), Palo Alto, CA 94304

[24]Catalog No. AX0116-6, EM Science, An Affiliate of MERCK KGaA, Darmstadt, Germany

[25]Catalog No. C-1276, Sigma Chemical Co., St. Louis, MO 63178

extraction. The solution had been adjusted to allow for moisture content from the sample and cysteine solution. For the total pigment measure, one third of the water in the initial acetone solution was replaced by concentrated HCl[26]. About 25 g of meat was ground in a beaker using Biomixer Blender[27] at 7000 rpm speed. Next, 5 g of the sample was placed in a 50 ml glass tube containing 10 ml of acetone solution. The sample was then homogenized (6000 rpm) to a smooth paste. The remaining 12 ml of acetone solution was added and the whole sample was re-homogenized. The homogenized sample was kept in the dark for 1 h before the absorbance measurement. After 5 minutes of intermittent mixing, the sample was filtered through a Whatman No. 42 filer paper[28] and re-filtered again through a 0.45 $\mu$m Puradisc filter device[29] with syringe to remove any remaining suspended particles. Absorbance at 540 nm for nitrosopigment and 640 nm for total pigment was measured by a HP Spectrophotometer using a 1-cm cell against 80% acetone or acetone/HCl solution as a blank.

**Statistical Analyses**

The experimental design was a partially confounded $2^4$ factorial design. Only eight of the sixteen treatments could be evaluated per day; therefore, the experiment was arranged into two blocks, thus introducing a confounding effect. The plant, replication, and treatments effects for response variables (pH, ORP, metmyoglobin, nitrosopigment, and total pigment) were analyzed using the MANOVA option of the general linear models (GLM) procedures of SAS (SAS Institute, 1989). The main effects and all contrasts (treatment combinations) were tested using a Wilks'

[26]J.T. Baker, A division of Mallincrodt Baker, Inc., Phillisburg, NJ 08865
[27]Catalog No. 11-504-204, Fisher Scientific, www.fishersci.com
[28]Catalog No. , Whatman Inc., Clifton, NJ 07014
[29]Catalog No. 6785-2504, Whatman Inc., Clifton, NJ 07014

Lambda. If the Wilks' Lambda test was significant, univariate analyses were performed using ANOVA F-tests for each variable at alpha = 0.05. If the ANOVA F-test was significant for a given variable, planned comparisons between treatments were done using the LSD method (Fisher protected LSD). Otherwise, planned comparisons with the Bonferroni method were performed using LSMEANS option with PROC GLM procedure of SAS. The three tested color groups in the experiment were subjected to individual statistical analyses.

Pink defect data were coded by assigning 1 to a positive response when a* $\geq$ 3.8 (pink), and 0 when a* $<$ 3.8 (not pink). The stepwise forward selection option of PROC LOGISTIC procedure was used with a DESCENDING option for model event to relate the occurrence of pinking to raw meat conditions in the following logistic function:

$$P(1/x) = \left( \frac{\exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n)}{1 + \exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n)} \right)$$

where $P(1/x)$ is the probability of the occurrence of pinking; $x_1, x_2, \cdots, x_n$ are raw meat endogenous conditions; $\alpha, \beta_1, \beta_2, \cdots, \beta_n$ are the parameter estimates associated with the model terms. A significance level of 0.15 was used for the inclusion criterion.

The predictive value of the developed logistic model was evaluated by analyzing an independent set of samples from one of the processing plants for three color groups. Sample were subjected to the same treatment and analyses as in the main experiment.

## 4.4 Results

### Significance of Plant and Replication

Multivariate analysis of variance showed significant (P $<$ 0.05) effect of all tested treatment combinations based on the Wilks' Lambda. Therefore, further analyses, using ANOVA and F statistics, were performed for each independent variable

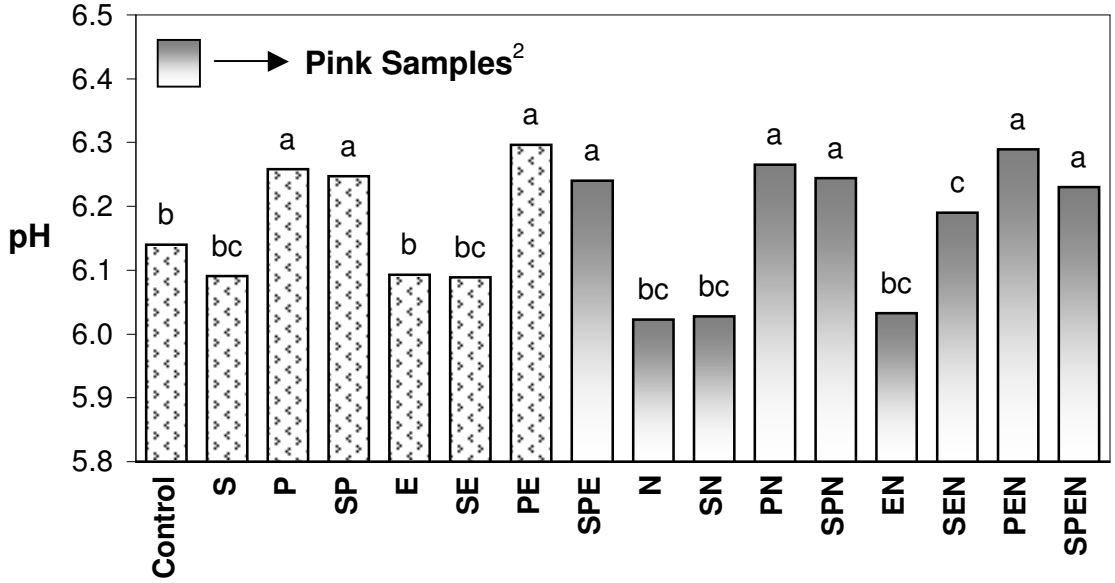
separately. There was no significant effect ($P > 0.05$) of replication per plant when all independent variables were tested using a 'replication within plant effect' as a divisor (data not shown). Also, the plant effect was not significant ($P > 0.05$) when tested using a residual error. Therefore, in subsequent analyses, the data were pooled across plants and replications.

**pH Conditions**

The three color groups responded differently to the treatment combinations. There was a significant effect ($P < 0.05$) of tripolyphosphate and sodium chloride on raw pH of samples from normal group (Figure 4.1-A). Samples injected with tripolyphosphate alone or in combination with other ingredients had the highest pH values, ranging from 6.230 to 6.296. These values were significantly different from those of other treatment combinations. High initial pH in raw meat, however did not always correspond exactly to pinking after cooking. Among all the samples in normal group with high pH, with one exception, only those containing sodium nitrite exhibited pinking. Fillets injected with a combination of sodium chloride, tripolyphosphate, and erythorbate having high raw meat pH=6.240 also exhibited pinking after cooking. Among the normal group samples with the pink defect, those injected with tripolyphosphate had significantly higher pH values compared to samples with no tripolyphosphate.

A similar response to tripolyphosphate was observed in the light group. Light group samples containing tripolyphosphate showed pH values ranging from 6.153 to 6.248 (Figure 4.1-B). In the light group, pinking was not observed in samples with high raw meat pH except when sodium nitrite was also present in the injection solution. Low pH value of the control sample in this group (5.920) indicated that increased pH up to 6.248 was associated mainly with the presence of sodium

A. Normal group




B. Light group

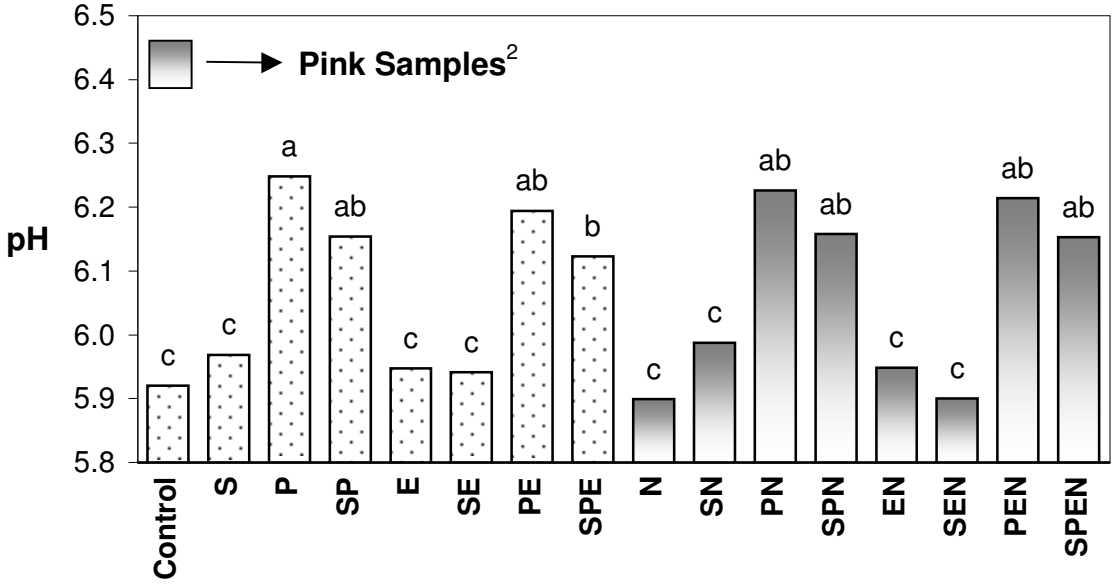



Figure 4.1: Effect of treatment combinations[1] on pH in raw chicken breast fillets

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-f}$Bars with no common letters differ significantly ($P < 0.05$)

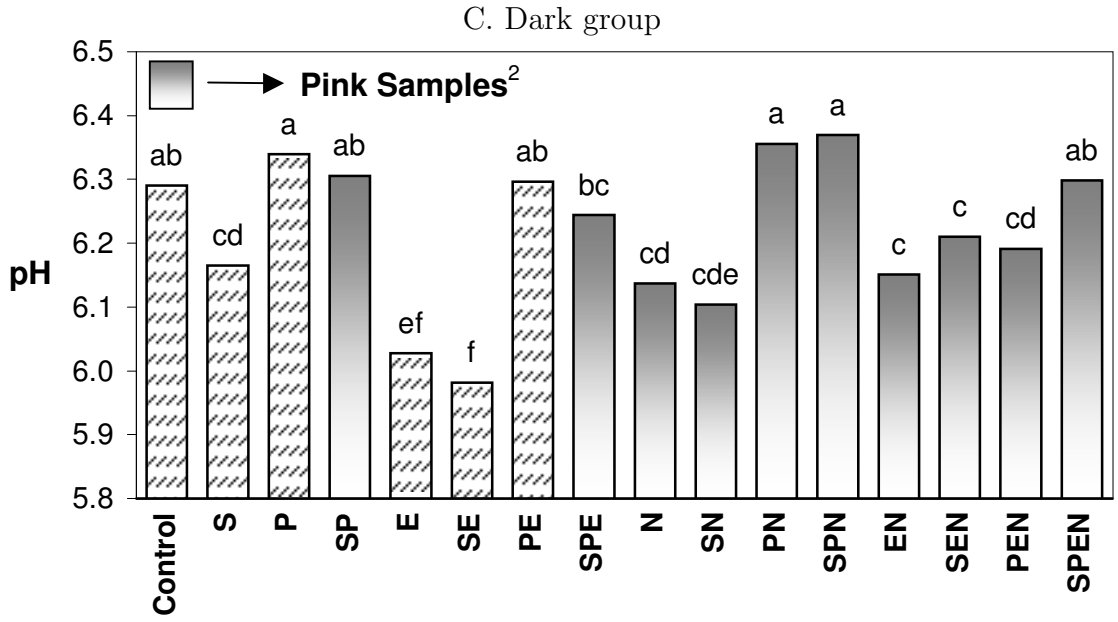



Figure 4.1: Cont.

tripolyphosphate in the injection solution. Among pink cooked fillets, those containing tripolyphosphate showed significantly higher pH values (5.135–6.226). The dark group showed marked variation in raw meat pH (5.982 – 6.356) in response to injections (Figure 4.1-C). Pinking occurred in all samples containing sodium nitrite; thus, high pH caused by tripolyphosphate was not always the determining factor. The significant positive effect of tripolyphosphate on pH in the dark group was accompanied by the significant negative effect of sodium erythorbate. Not all the samples with the pink defect in the dark group had a significantly higher raw meat pH than samples with no pinking, and not all of the high pH samples exhibited pinking after cooking. In two instances, pinking occurred in the absence of sodium nitrite: the combination of sodium chloride and tripolyphosphate and the combination of sodium chloride, tripolyphosphate, and erythorbate. Interestingly, in these

two instances, pH values (6.305 and 6.244) of raw samples were not significantly different from the control (6.290).

### Oxidation-Reduction Potential (ORP)

The oxidation-reduction potential was expressed as a change in ORP relative to the control (a "zero" value). The magnitude of change was obtained by subtracting the ORP of the control from that of the sample; the more negative the ORP value, the greater was the reducing condition. Initial ORP values of raw muscles ranged from $-62.80$ to $-66.40$ for the light group, from $-58.10$ to $-177.02$ for the normal group, and from $-102.87$ to $-158.40$ for the dark group. There was a significant effect ($P < 0.05$) of sodium erythorbate on ORP changes in the normal, light, and dark groups (Figures 4.2-A,B,C). Only the treatment combination of sodium chloride, tripolyphosphate, and erythorbate in the normal group exhibited both pinking and a significant negative change in ORP (Figure 4.2-A).Other treatment combinations in the normal group resulting in pink defect included: (1) samples with sodium nitrite and positive ORP change; and (2) samples with sodium nitrite and negative ORP change. The light group was most prone to ORP changes caused by the addition of sodium erythorbate (Figure 4.2-B). However, the light group did not show more pinking except when samples were injected with nitrite or with both nitrite and erythorbate. As presented in Figure 4.2-C, not all the samples from the dark group with pinking exhibited low ORP; substantial variation in ORP, not necessarily related to pinking, occurred in this group before cooking. The combination of sodium chloride, tripolyphosphate, and erythorbate had caused pinking and lower ORP. On the other hand, a combination of sodium chloride with tripolyphosphate effected pinking while demonstrating a positive ORP change (3.49); this indicated that the sample had an ORP value even higher than that of the control.

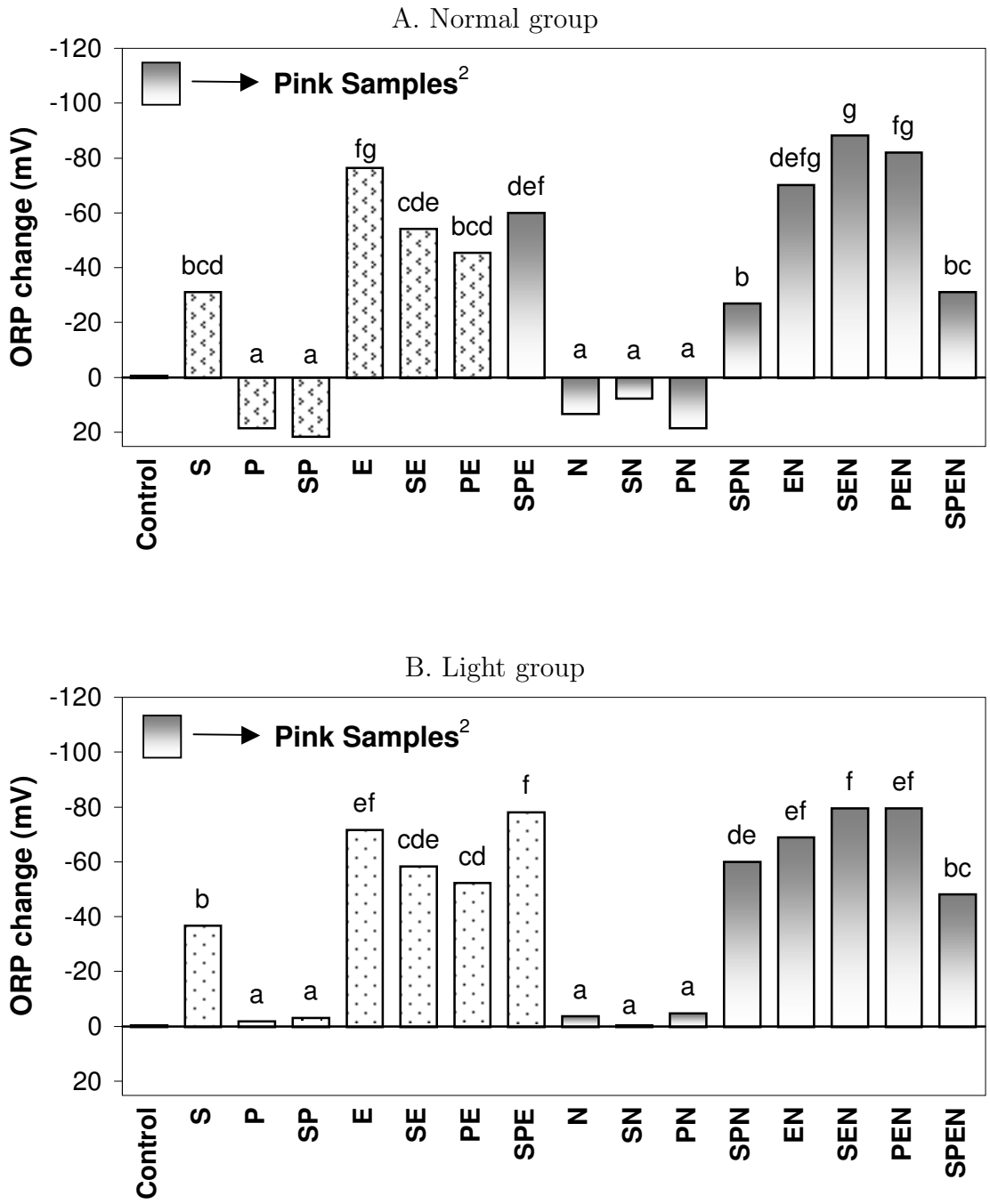



Figure 4.2: Effect of treatment combinations[1] on oxidation-reduction potential (ORP) in raw chicken breast fillets

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-g}$Bars with no common letters differ significantly ($P < 0.05$)

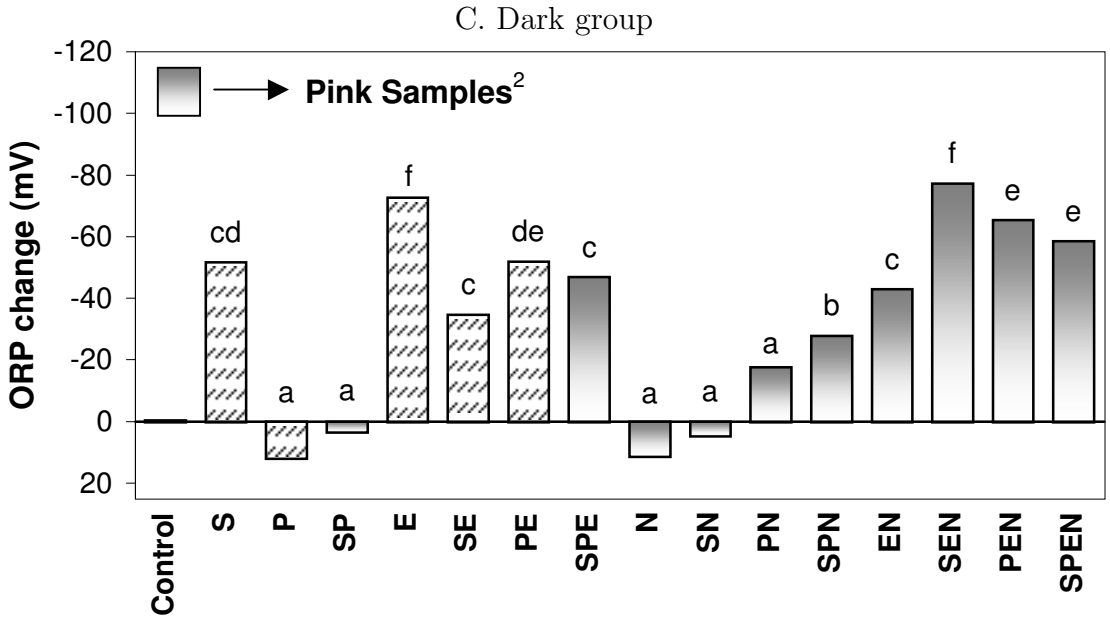



Figure 4.2: Cont.

**Percent Metmyoglobin**

Widely varying percentages of metmyoglobin was present in raw fillets from the normal group with pinking, thus indicating that the percentage of metmyoglobin in raw meat was not a good indicator of pinking (Figure 4.3-A). A similar trend was observed in the light group (Figure 4.3-B). Among normal group samples with pinking defect only the combination of sodium chloride, tripolyphosphate and erythorbate, and the combination of tripolyphosphate, erythorbate and nitrite showed a significantly lower content of metmyoglobin, 67.01 and 65.74, respectively (Figure 4.3-A). However, only the latter combination caused significant decrease in metmyoglobin compared to the control (69.97). The other samples from the normal

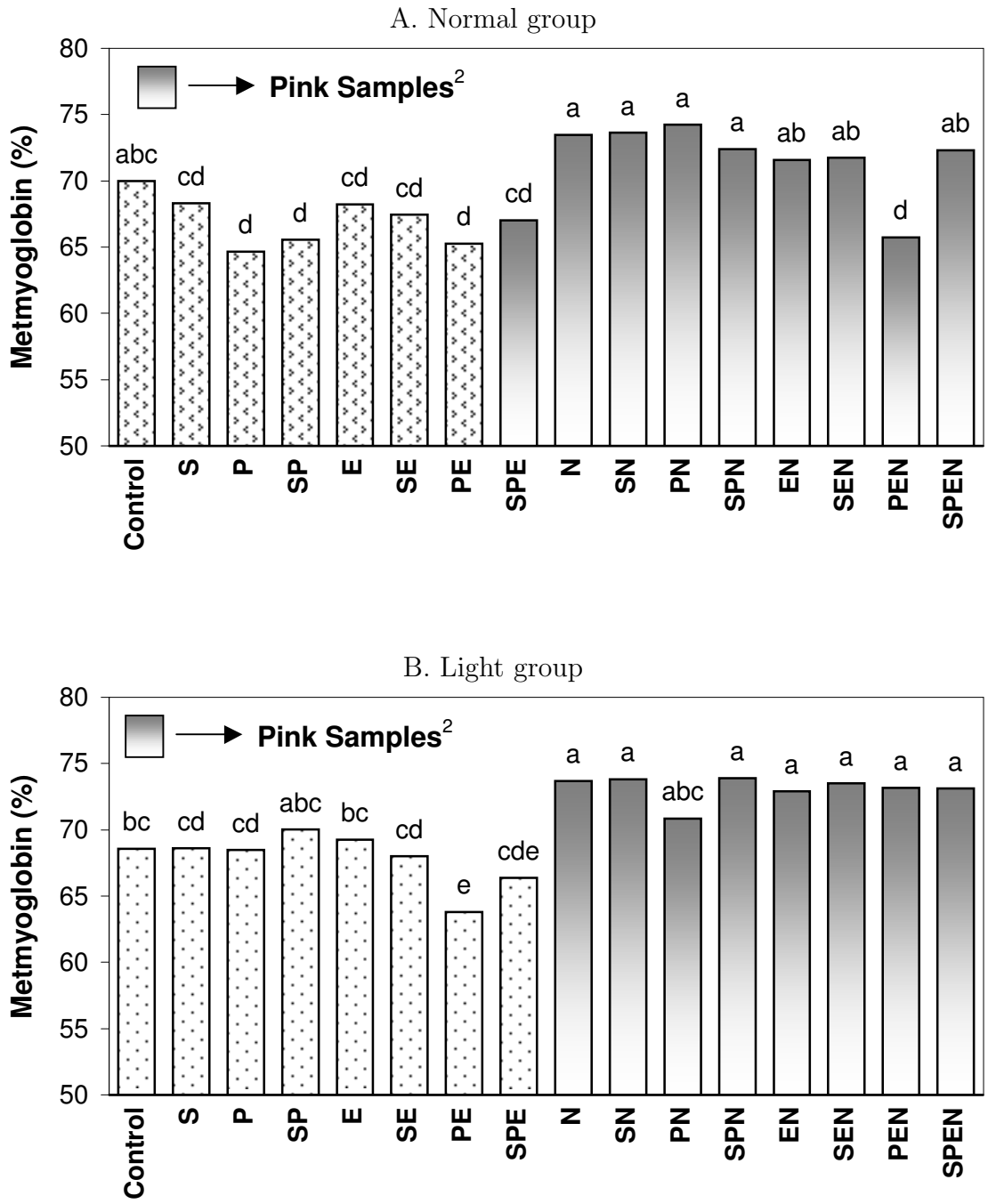



Figure 4.3: Effect of treatment combinations[1] on percentage of metmyoglobin in raw chicken breast fillets

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-f}$Bars with no common letters differ significantly ($P < 0.05$)

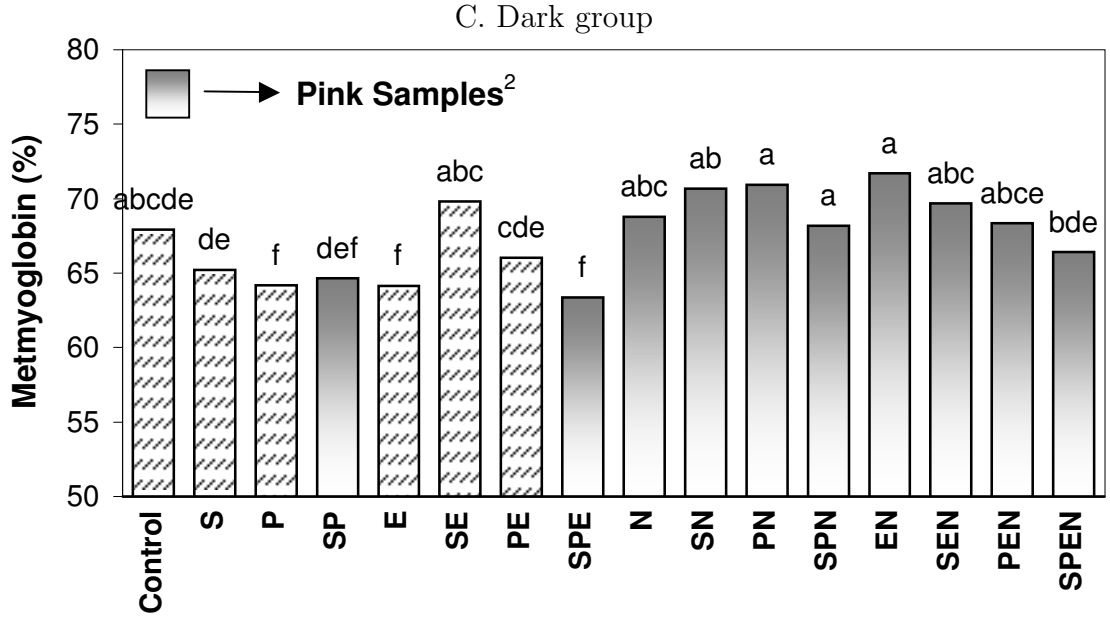



Figure 4.3: Cont.

group that exhibited pinking had a percentage of metmyoglobin that was not significantly different than that of the control sample. All the samples in the light group, which were pink after cooking, had a significantly higher percentage of metmyoglobin compared to the control except in one instance: the combination of tripolyphosphate and nitrite (Figure 4.3-B).There was even more variation in the percentage of metmyoglobin in the dark group (Figure 4.3-C). Sample with pink defect from the dark group injected with the combination of sodium chloride, tripolyphosphate, and erythorbate had a significantly lower percentage of metmyoglobin (63.35) than the rest of the pink fillets (66.41–71.71). However, there was one exception — samples with pink defect injected with sodium chloride and tripolyphosphate (64.66%).

### Nitrosopigment Content

Nitrosopigment content showed a significant and positive effect on pinking in all three color groups. When the nitrosopigment content of raw meat was estimated at a level higher than 1 ppm, all of the cooked fillets exhibited pinking (Figures 4.4-A,B,C).

In addition, a combination of sodium chloride, tripolyphosphate, and erythorbate caused pinking of the normal and dark groups (Figures 4.4-A,C). Only the dark group exhibited pink defect when injected with the combination of sodium chloride with tripolyphosphate. Pinking in those treatment combinations lacking sodium nitrite were probably affected by other induced conditions such as pH and reducing conditions. Surprisingly, sodium nitrite in combinations with other ingredients caused a two- to threefold increase in the nitrosopigment in fillets when compared to samples injected with sodium nitrite only. It was concluded that sodium chloride, tripolyphosphate, and erythorbate formed favorable conditions for nitrosopigment formation through met- or myoglobin combination with both naturally present and added nitrite.

### Total Pigment

The pigment content, expressed as ppm of hematin, did not show specific trends toward pinking (data not shown). Hematin concentration ranged from 12.43 to 31.2 ppm in the normal group, from 13.34 to 26.84 ppm in the light group, and from 10.62 to 15.61 ppm in the dark group. Substantial variation in ppm hematin in the results of different treatment combinations could not be explained and no firm conclusions were drawn with regard to the pink defect.

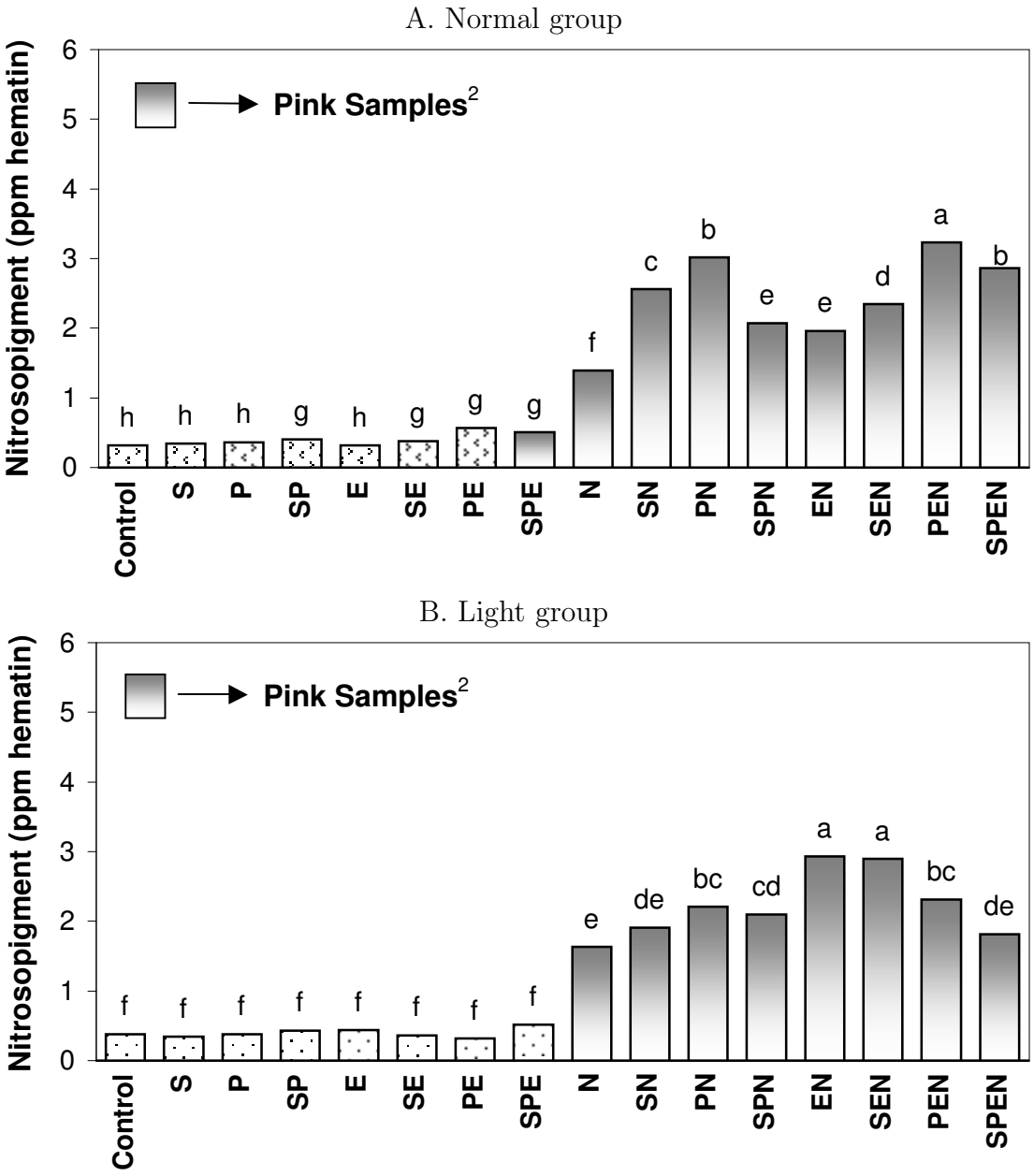



Figure 4.4: Effect of treatment combinations[1] on nitrosopigment content in raw chicken breast fillets

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-h}$Bars with no common letters differ significantly ($P < 0.05$)

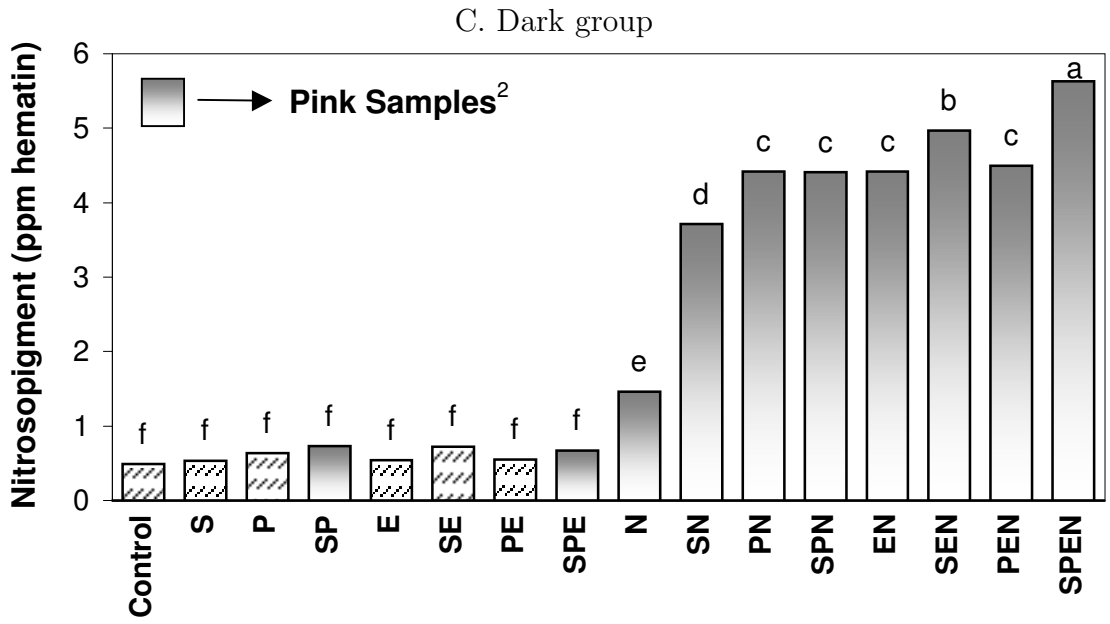



Figure 4.4: Cont.

Table 4.1: Results of logistic regression conducted to identify raw meat variables critical for pink defect in cooked meat expressed as probability of the pink defect[1]

| Muscle Color group | Source | df | Parameter Estimate | Standard Error | Wald Chi-Square | P > ChiSq | Odds Ratio Estimate |
|---|---|---|---|---|---|---|---|
| Light | Intercept | 1 | -16.5839 | 17.5338 | 0.8946 | 0.3442 | . . . |
| | Nitrosopigment[2] | 1 | 15.9295 | 16.3764 | 0.9489 | 0.3300 | > 999.999 |
| Normal | Intercept | 1 | -9.0613 | 4.0392 | 5.0327 | 0.0249 | . . . |
| | Nitrosopigment | 1 | 11.1729 | 5.6026 | 3.9770 | 0.0610 | > 999.999 |
| | ORP[3] | 1 | -0.0418 | 0.0318 | 1.7269 | 0.1888 | 0.959 |
| Dark | Intercept | 1 | -53.6647 | 27.9156 | 3.6956 | 0.0546 | . . . |
| | Nitrosopigment | 1 | 8.7565 | 3.5405 | 6.1170 | 0.0134 | > 999.999 |
| | pH | 1 | 7.5334 | 4.3809 | 2.9570 | 0.0855 | > 999.999 |

[1]Significance, $\alpha = 0.15$
[2]Nitrosopigment, expressed as ppm of hematin
[3]Oxidation-Reduction Potential change relative to the control
$\cdots$ = Not applicable

**Logistic Regression Analysis**

The parameter estimates, the Wald chi-square statistic, and the odds ratio estimates for all three color groups are presented in Table 4.1. As expected, the logistic model for the light group resulted in a complete separation of data points. This is attributed to the presence of sodium nitrite as one of the ingredients in the injection solution leading to pinking defect. All of the pink samples had more than 1 ppm of nitrite. Therefore, the only raw meat variable that met the 0.15 significance level of entry into the model was nitrosopigment content. Based on the likelihood ratio chi-square test, at least one of the independent variables in the model was significant ($P< 0.0001$). The Wald statistic showed no significance with regard to the intercept and the nitrosopigment content. However, it was decided to keep it in the model. Based on the nitrosopigment parameter estimate, the odds that the dependent variable will cause pinking in the light group reached infinity. This situation was expected since the nitrosopigment concentration in the raw meat was the only variable determining the presence of a pink defect in the light group.

There were two variables, nitrosopigment content and oxidation-reduction potential change (ORP), that entered the model at a 0.15 significance level for the normal group. Analysis of model parameters estimates showed that when the nitrosopigment concentration increased by 1 unit, with the ORP variable remaining unchanged, the odds would increase by a factor of $e^{11.17}$, that is indefinitely. Considering the odds ratios corresponding to the ORP parameter in the model for the normal group, low and negative values of the estimate indicated that there was actually less chance of having a pink defect given a one unit increase in ORP change. Even though the ORP parameter was not significant ($P > 0.15$) in the model based on the Wald statistic, it was decided to keep it in the model as valuable information. When the logistic function was fitted to the data from the dark group, significance was found

for the intercept, pH, and nitrosopigment content. Both the pH and nitrosopigment variables showed that the probability of pinking increases with higher pH and higher nitrosopigment content. The odds ratios estimated at $e^{7.53} = 1863$ for pH and $e^{8.75} = 6310$ for nitrosopigment indicated that the odds of occurrence of pink defect per unit increase increased by a factor of 1863 for pH content and by 6310 for nitrosopigment content, respectively. The c statistic is a rank correlation index computed from pink/non-pink classification table (SAS, 1995). The closer the value of c approaches 1 the better the predictability of the logistic regression equation. The light group logistic equation with its sole predictor variable and with complete data separation had a c value equal to 1, as expected (data not shown). The normal and dark groups also showed a high level of differentiation between pink and non-pink events with group values of $c = 0.987$ and $c = 0.968$, respectively.

A classification table was used as a measure of model accuracy for each color group. The observed values for the pink defect and the predicted pinking (at a cutoff value of $p = 0.5$) were cross classified (Table 4.2). The logistic function for the light group correctly classified 63 of the 64 observations (98.4%). Since 31 of the 32 pink events were correctly predicted in the light group, the sensitivity for the model was 96.9%. Furthermore, the model also correctly classified all the non-pink samples yielding a specificity of 100%. None of the non-pink samples from the light group were falsely classified as pink samples (false positive). One pink sample was incorrectly classified as non-pink yielding a 3% false negative rate. The logistic function correctly classified samples from the normal group at 90.6%. There were two non-pink samples which were falsely predicted to be pink and four samples with pink defect that falsely predicted to be non-pink. Therefore, this model predicted the occurrence of pinking with 88.9% accuracy. More pink samples from the normal group were incorrectly predicted to be non-pink (false positive error) than non-pink samples were predicted to be pink (false negative error). The logistic model showed the same percentage

Table 4.2: Levels of correct classification from the logistic model (at a cutoff value of p=0.50) for light, normal, and dark groups

| | Correct | | Incorrect | | Percentages | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Muscle color group | Pink | Not Pink | Pink | Not Pink | Correct | Sensitivity[1] | Specificity[2] | False Positive[3] | False Negative[4] |
| Light | 31 | 32 | 0 | 1 | 98.4 | 96.9 | 100.0 | 0.0 | 3.0 |
| Normal | 32 | 26 | 2 | 4 | 90.6 | 88.9 | 92.9 | 5.9 | 13.3 |
| Dark | 36 | 22 | 2 | 4 | 90.6 | 90.0 | 91.7 | 5.3 | 15.4 |

[1]Ratio consisting of the number of correctly classified pink over the total number of pink
[2]Ratio consisting of the number of correctly classified non-pink over the total number of non-pink
[3]Proportion of predicted pink responses that were observed as non-pink
[4]Proportion of predicted non-pink responses that were observed as pink

for the dark group as for the normal group in predicting pinking. However, the proportion of pink samples that were predicted to be pink (sensitivity) was higher in the dark group compared to the normal group. On the other hand, the specificity of the model for the dark group was lower than that of the normal group. The model for the dark group produced more false negative errors than did the normal group model.

The logistic models for the three color groups correctly predicted more than 90% of the pinking in cooked samples. After transforming $logit(p)$ into probability of pinking in the light, normal, and dark group, logistic regression equations were as follows:

*Pink defect probability for the light group*

$$= \frac{\exp(-16.58 + 15.95x_1)}{1 + \exp(-16.58 + 15.95x_1)} \tag{4.1}$$

*Pink defect probability for the normal group*

$$= \frac{\exp(-9.06 + 11.17x_1 - 0.041x_3)}{1 + \exp(-9.06 + 11.17x_1 - 0.041x_3)} \tag{4.2}$$

*Pink defect probability for the dark group*

$$= \frac{\exp(-53.66 + 8.75x_1 + 7.53x_2)}{1 + \exp(-53.66 + 8.75x_1 + 7.53x_2)} \tag{4.3}$$

where $x_1$ - Nitrosopigment (ppm); $x_2$ - pH; $x_3$ - Oxidation-reduction potential change relative to the control.

A new independent data set, from one plant with one replication, was used for model validation. The models (Eq 4.1, 4.2, 4.3), generated with the SAS LOGISTIC procedure, were used for verification and to generate the predicted values for observations from the independent data set. These predicted values were then compared with observed pinking in samples from the independent data set and cross tabulated in the two-way frequency table using a FREQ procedure from SAS. In the TABLES statement, Observed-Pink*Predicted-Pink specified a table where the columns are the observed pink events and the rows are the predicted pink observations. Table 4.3 presents the cross tabulation of Observed Pink and Predicted Pink. In the first cell, 100% of those samples with observed pink were predicted as pink in the light and normal groups, and 90% of pink samples were predicted as pink in the dark group. There was significant evidence of an association between observed pink samples from independent data set and those predicted as pink (data not shown). Fisher's exact test yielded a two-sided P-value of less than 0.001 for the light, normal, and dark groups. The results with an independent set of data confirmed the validation of the three logistic models built for the light, normal, and dark groups.

Table 4.3: Cross Tabulation of Observed-Pink by Predicted-Pink[1]

| Muscle Color Group | Observed Pink | | Predicted Pink | | |
|---|---|---|---|---|---|
| | | | YES | NO | Total |
| Light | YES | *f* | 8 | 0.00 | 8 |
| | | *tp* | 50.00 | 0.00 | 50.00 |
| | | *rp* | 100.00 | 0.00 | |
| | | *cp* | 100.00 | 0.00 | |
| | NO | *f* | 0 | 8 | 8 |
| | | *tp* | 0.00 | 50.00 | 50.00 |
| | | *rp* | 0.00 | 100.00 | |
| | | *cp* | 0.00 | 100.00 | |
| | Total | *f* | 8 | 8 | 16 |
| | | *tp* | 50.00 | 50.00 | 100.00 |
| Normal | YES | *f* | 9 | 0 | 9 |
| | | *tp* | 56.25 | 0.00 | 56.25 |
| | | *rp* | 100.00 | 0.00 | |
| | | *cp* | 100.00 | 0.00 | |
| | NO | *f* | 0 | 7 | 7 |
| | | *tp* | 0.00 | 43.75 | 43.75 |
| | | *rp* | 0.00 | 100.00 | |
| | | *cp* | 0.00 | 100.00 | |
| | Total | *f* | 9 | 7 | 16 |
| | | *tp* | 56.25 | 43.75 | 100.00 |
| Dark | YES | *f* | 9 | 1 | 10 |
| | | *tp* | 56.25 | 6.25 | 62.50 |
| | | *rp* | 90.00 | 10.00 | |
| | | *cp* | 14.29 | 100.00 | |
| | NO | *f* | 0 | 6 | 6 |
| | | *tp* | 0.00 | 37.50 | 37.50 |
| | | *rp* | 0.00 | 100.00 | |
| | | *cp* | 0.00 | 85.71 | |
| | Total | *f* | 9 | 7 | 16 |
| | | *tp* | 56.25 | 43.75 | 100.0 |

[1]In each cell the values printed under the cell count (frequency - $f$) are the table percentage ($tp$), row percentage ($rp$), and column percentage ($cp$)

## 4.5 Discussion

There are not many research reports available that attempt to relate raw meat conditions to pinking after cooking. The high pH (6.25) and abnormally red chicken breast muscles because of preslaughter factors (struggle, and excitation of birds) has been reported by Ngoka et al. (1982). They also concluded that when excitation and struggle occur in the prior to birds slaughter it may lead to some pinking problems. Janky and Froning (1973) reported that a pH increase from 5.5 to 6.9 is associated with lower percentage of myoglobin denatured and may be related to pinking. In addition, Young et al. (1996a) related higher pH (6.10–6.46) of raw turkey breast meat caused by a short post-mortem time to the significantly increased CIE a* values in raw meat. Also according to Allen et al. (1998) there is a correlation between raw meat pH and raw meat color. Most of the information in regard to oxidation-reduction potential present in meat represents the results that were obtained after cooking as an explanation what conditions were present at the time the defect occurred. However, no information is available about observed ORP changes in raw meat in relation to pinking. Other researchers have studied the pinking defect caused by nitrite levels equal to or much greater than that used by us. More than 200 ppm of nirite-nitrogen present in chilling water was related to the increased redness of meat after cooking (Mugler et al., 1970). Also, significant color development occurred when chicken carcasses were held in water or ice containing 3 ppm of nitrite (Nash et al., 1985). According to Froning et al. (1969) when birds were fed with diet containing 25 or 50 ppm of sodium nitrite the Gardner $a_L$ value were reported to be more than 4.0. However, authors did not report nitrite levels in raw meat. Ahn and Maurer (1985) found in their study that 1 ppm of added nitrite caused pinking in cooked turkey breast. Our study also clearly indicated that 1 ppm of sodium nitrite in the raw meat is related to pinking.

## 4.6 Conclusions

The induced in situ conditions used in this study played a significant role in the occurrence of pinking. As expected, none of the ingredients alone, except sodium nitrite, significantly induced pinking. As reported in the literature, a small, almost residual, amount of nitrite may be present in meat depending on the preslaughter factors and processing conditions. Therefore, the probability of pinking is high whenever sodium nitrite ($>$ 1 ppm) is present in meat before cooking. In the absence of nitrites, the other commonly used ingredients come into play: sodium chloride, sodium tripolyphosphate, and sodium erythorbate. These ingredients are well known to affect (1) solubility of meat proteins and their resistance to denaturation, (2) the increase in meat pH, and (3) the increase in reducing conditions. In the present study, we showed that it is unlikely that a change in only one of the above conditions would produce pinking. However, the combination of sodium chloride, tripolyphosphate, and erythorbate induced significant changes in pH, ORP, and percent of metmyoglobin present. Values of pH higher than 6.2 that were accompanied by increased reducing conditions ($-100$mV to $-200$mV) and metmyoglobin concentration at about 67% in raw meat resulted in simulated pink defect in the normal muscle group. Neither pH increase from 5.920 to 6.248 nor induced reducing conditions produced pinking in the light group. High initial pH (6.290) of the muscles from the dark group that was further increased by presence of sodium tripolyphosphate resulted in simulated pinking even without significant decrease in ORP values. The natural variation in raw color of breast muscles also showed an effect on the occurrence of pinking, with the light group being least affected and the dark group most affected. The logistic regression and its validation as presented in this study demonstrated its use as a predictor of pinking based on the raw meat conditions. In addition, nitrosopigment content in raw meat, pH $>$ 6.2, and reducing conditions

(ORP $< -100$mV) were shown to be the most important factors in pinking. These results are important in assisting the poultry industry in (1) assessing the potential for pinking and (2) in investigating new procedures to prevent and eliminate the pink defect.

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# Chapter 5

# Cooked Chicken Breast Meat Conditions Related to Simulated Pink Defect[1]

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To be submitted to *Journal of Food Science*

## 5.1 Abstract

The objective of this research was to determine the effect of induced in situ conditions (sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite) on the pink defect in chicken breast meat. The simulation of the pink defect (based on subjective pink threshold) was achieved both with and without sodium nitrite. Simulated pink defect was significantly affected by lightness of raw muscles, increased pH, and decreased redox potential. The simulation can aid in establishing pink threshold for poultry industry and in developing alternate processing methods to eliminate any pink defect.

*Keywords:* pink defect, cooked chicken white meat, in situ conditions

## 5.2 Introduction

The pink color defect (pinking, pinkness, or pink tinge) in cooked poultry white meat is perceived by consumers as an undercooked and unsafe to eat product. The USDA Food Safety and Inspection Service regulations require that heat-processed poultry products sold as ready-to-eat must be cooked to 71.1°C (FSIS USDA, 1999). Notwithstanding this requirement, the pink color defect may be responsible for more than $10,000,000 of annual losses in Georgia, through rejection, rework, and condemnation (Reynolds 2002). More than 1.25 billion broilers are slaughtered and processed into food products each year in Georgia alone (USDA-NASS 2002). With such huge numbers, even a small defect can translate into huge economic losses for the poultry industry. The well documented factors associated with pinking have been related to the presence of three pigment classes: 1) undenatured myoglobin or oxymyoglobin (Tappel 1957; Bernofsky and others 1959; Trout 1989), 2) reduced globin hemochromes of well-cooked meats (Trout 1989), 3) nitrosyl hemochrome, the pink pigment of cured meat (Froning and others 1969; Mugler and others 1970; Ahn and Maurer 1987, 1989a; Fleming and others 1991), 4) carboxymyoglobin (Cornforth and others 1998; Nam and Ahn 2002). In addition, a hemo-protein found in a muscle's mitochondria, cytochrome c, can also play a role in pinkness because of its high stability and reactivity under different conditions (Ahn and Maurer 1989b; Girard and others 1990). A higher pH decreases myoglobin denaturation at given temperatures and allows greater reactivity of pigments (Janky and Froning 1973; Trout 1989; Ahn and Maurer 1990b; Young and others 1996a, 1996b; Hunt and others 1999). An important factor in pink color formation is the oxidation-reduction potential (ORP) which determines the state of the hemochrome ($Fe^{2+}$)/hemichrome ($Fe^{3+}$) iron in globin hemochromes; ORP is the determining factor in the ability of pigments to combine with or tie up molecules (Akoyonouglu and others 1963; Cornforth and

others 1986; Ahn and Maurer 1989b; Ghorpade and Cornforth 1993). The ORP is a function of both pH and the presence of reductants. In addition, high reducing conditions have been associated with unstable meat color (Cornforth and others 1986; Ahn and Maurer 1989a, 1990a). It has been suggested that incidental nitrate/nitrite contamination (preslaughter factors, processing equipment, water supply) is the most probable factor responsible for the pink defect in cooked poultry products (Mugler and others 1970; Nash and others 1985; Ahn and Maurer 1989a; Heaton and others 2000). All of the factors seem to influence specific in situ conditions such as pH, reducing conditions, and the chemical state and reactivity of pigments. In our laboratory we hypothesized that simulation of the pink defect would serve as a research tool to investigate procedures for prevention or elimination of the pink defect. The specific research objectives involved (1) examining the effects of treatments that would induce measurable changes in the in situ conditions of cooked meat, and (2) to study the relationship between induced muscle conditions and the pink defect.

## 5.3 Materials and Methods

The material for the experiment was boneless, skinless, chicken breast fillets obtained from processing plants. Fillets were selected based on CIE (C.I.E. 1978) L* lightness into three color groups: lighter than normal (L* > 50), normal (47 < L* < 50), and darker than normal (L* < 47) (Fletcher 1999a,b,c; Fletcher and others 2000). Sorting of the fillets included visual appearance at the deboning line or at the beginning of a "further processing" line followed by verification using a Hunter Lab reflectance colorimeter (MiniScan/XE 45/0-L, Hunter Associates laboratory, Inc., Reston, VA). Fillets were selected based on the medial surface (bone side) lightness. After packing in ice, samples segregated according to color group (light, normal, or dark) were transported to the laboratory.

The experiment included two processing plants with two replications each. Because of seasonal variation in the material, each sampling for replication was separated by a period of at least 4 months. A total of 960 samples were collected for the experiment. Two hundred forty fillets (eighty for each of the three color groups) associated with each replication and plant could not be collected in a single visit due to unavailability of the desired number of breast fillets in each color group at a given time. Therefore, 240 fillets were collected per replication during two or three visits to each of the two processing plants.

All breast fillets, 24 h postmortem, were vacuum packed (Division of KOCH Supplies, Inc., Kansas City, MO) into sampling polyethylene bags and labelled individually, frozen and stored at −18°C up to 3 weeks before the experiment. After adequate thawing at 4–5°C (approximately 10–12 h), samples were ready for the experiment. There were four treatment factors, sodium chloride (1g/100g meat) (Fisher, www.fishersci.com), sodium tripolyphosphate (0.5g/100g meat) (Sigma Chemical Co., St. Louis, MO), sodium erythorbate (0.0546g/100g meat) (Aldrich Chemical Company, Inc., Milwaukee, WI), and sodium nitrite (1 ppm) (Sigma Chemical Co., St. Louis, MO). The level of each factor was chosen according to standard regulations and common industry applications. Two levels of factors were used — present and not present — resulting in sixteen treatment combinations. The injection method (12% of meat weight basis) was used to incorporate ingredients into the samples. The ingredients were prepared in stock solutions: 0.1% sodium nitrite, 10% sodium tripolyphosphate, and 1% sodium erythorbate. The 12% injection was achieved by combining the stock solutions to obtain the desired final volume and concentrations of 0.5% sodium tripolyphosphate, 0.0546% sodium erythorbate and 1 ppm sodium nitrite. Sodium chloride was added as dry ingredient to the injection solution according to the weight of a given sample. Sixty-four injection solutions were required (4 muscles x 16 treatment combinations). Samples were injected using

a 35 ml syringe (Sherwood Medical Company, St. Louis, MO) equipped with a stainless steel 16 hole spray needle (KOCH Supplies, N. Kansas City, MO). Injected samples were packed individually in moisture-impermeable polyethylene bags, tumbled, and refrigerated for equilibration overnight (12 h) at 4–5°C before further experimentation.

A laboratory made small table-top tumbler (O.D 40 cm, two equally spaced webs) was used for the tumbling process. During each of the eight tumbling occasions, four individually packed muscles (∼25% load) from the same treatment were tumbled for 15 min at 45 RPM with 1.0 kg of ice in order to keep the product temperature at about 0°C. The following morning, two of the four fillets were subjected to cooking. The two fillets were placed in aluminum mini broiler pans (HANDI-FOIL Corporation, Wheeling, IL) and covered with aluminum foil. Samples were cooked in a 167°C convection oven (GS LINDBERG/BLUE M, Blue Island, IL) to an internal temperature of 74°C. For the cooling process, samples were first placed in a temperature controlled laboratory where the temperature was maintained at 21°C. After first 45 min of cooling the samples to 48°C, samples were then cooled to 4.4°C by placing them in the walk-in environmental cooler maintained at 4°C. The final targeted temperature of the samples (∼4°C) was achieved within 4 h. The internal temperature of the product was monitored throughout the cooking and cooling process using HP BenchLink Data Logger 1.1 and data acquisition unit (Hewlett-Packard Company, Loveland, CO). K-type chromel-alumel thermocouples were inserted in the center of a cranial end of a muscle before cooking. One thermocouple was used per cooking pan.

**Identification of pinking**

The subjective pink threshold established previously (Holownia and others 2002a) at CIE a* = 3.8 (with L* ranging from 79 – 84 and b* from 10 – 16) was used in judging the existence of pinking in the cooked samples. The threshold had been produced by injecting samples from the normal group with 1% sodium chloride and sodium nitrite solutions (1, 2, 3, 4, and 5 ppm).

The experimental cooked samples were sliced lengthwise horizontally into halves. Immediately, color was objectively evaluated in the light booth (Illuminant D65), and an a* value was measured using a Hunter lab colorimeter. Eight measurements were taken on each fillet — four on each of the halves of the fillets with two readings in the anterior and two in the posterior portion of the muscle — rotating the samples 90° between measurements. The average value of eight measurements was used. The presence or absence of pinking was judged based on both visual and instrumental results.

**pH measurement**

The muscle pH of cooked meat was determined using the iodoacetate method of Jeaocke (1977). Approximately 25 g of meat tissue was removed from anterior portion of the fillet and ground. Two grams of each sample was homogenized (30 s, 3000 rpm) (Omni International, Inc., Gainesville, VA) with 25 ml of 5 mM iodoacetate (Sigma Chemical Co., St. Louis, MO) containing 150 mM KCl and neutralized to pH = 7.0 with few pellets of KOH (Sigma Chemical Co., St. Louis, MO). The pH of the meat slurry was measured with a pH meter (ORION, Model 525A+, Thermo Orion, Beverly, MA) and a combination pH electrode (Thermo Orion, Beverly, MA). Mean values of the duplicate observations were recorded.

**Oxidation-reduction potential (ORP)**

Twenty gram of ground cooked meat was rapidly homogenized with 30 ml of 0.1M sodium carbonate (Sigma Chemical Co., St. Louis, MO). A platinum combination redox electrode (Thermo Orion, Beverly, MA) was used to measure ORP of the meat slurry (3–5°C). ORP readings (mV) were recorded after 5 minutes of stabilization (Cornforth and others 1986).

**Percent of myoglobin denatured (PMD)**

Myoglobin was extracted from the raw and cooked meat using an ice-cold (0°C) 0.04 M phosphate buffer, pH=6.8 (Warris 1979). Twenty five grams of the raw or cooked sample was removed from the anterior portion of the fillet and ground. Next, 5 g of raw or 10 g of cooked samples was homogenized with 50 ml or 40 ml of buffer, respectively, at 10,000 rpm for 20 s. The homogenized samples were centrifuged (Beckman Model J2-21M, Rotor JA-14, Beckman Instruments, Inc., Palo Alto, CA) for 30 min at 15,000g at 4°C. The supernatant was then filtered through a Whatman No.1 filter paper. Absorbance of the raw and cooked filtrates was measured at 525, 572, and 700 nm (Krzywicki 1979) using a HP 8451A Diode Array Spectrophotometer (Agilent Technologies (Hewlett-Packard), Palo Alto, CA). Percent myoglobin concentration and percent myoglobin denatured (PMD) were calculated using the formulas (Trout 1989):

$$Myoglobin\ (mg/ml) = (A_{525} - A_{700})2.303(dilution\ factor)$$

$$PMD = \left[1 - \left(\frac{myoglobin\ after\ cooking}{myoglobin\ before\ cooking}\right)\right] 100$$

where, $A_\lambda = Absorbance\ at\ \lambda, nm.$

**Nitrosyl hemochrome and total pigment**

The Hornsey's method (Hornsey 1956) with modification of Pearson and Tauber (1984) was used for measuring nitrosyl hemochrome and total pigment concentration in cooked meat. Nitrosopigment and total pigment were extracted from 5 g of a sample using 22 ml 80% acetone (EM Science, An Affiliate of MERCK KGaA, Darmstadt, Germany) solution. To minimize pigment's fading, neutralized cysteine (Sigma Chemical Co., St. Louis, MO) solution (0.5%) was added to the sample in the amount of 0.5 ml before extraction. One third of the water in the initial (80%) acetone solution was replaced by concentrated HCl (J.T. Baker, A division of Mallincrodt Baker, Inc., Phillisburg, NJ) for the total pigment's extraction. Five gram of ground sample was homogenized (6000 rpm) first with 10 ml of acetone solution to a smooth paste. Next, the remaining 12 ml of acetone solution was added and the whole sample was re-homogenized. The homogenized samples for total pigment evaluation were kept in the dark for 1 h. The nitrosopigment samples were filtered through a Whatman No. 42 filer paper and re-filtered again through a 0.45 $\mu$m Puradisc filter device (Whatman Inc., Clifton, NJ) with syringe. Absorbance at 540 nm for nitrosyl hemochrome and 640 nm for total pigment was measured by a HP Spectrophotometer using a 1-cm cell against 80% acetone or acetone/HCl solution as a blank.

**Statistical Analyses**

The experimental design was a partially confounded $2^4$ factorial design. The block was the confounding effect because only eight of the sixteen treatments could be evaluated per day and the experiment was arranged into two blocks. The plant, replication, and treatments effects for response variables (pH, ORP, metmyoglobin, nitrosopigment, and total pigment) were analyzed using the MANOVA option of the

general linear models (GLM) procedures of SAS (SAS Institute 1989). The Wilks' Lambda was used to test a significance of all contrasts. Univariate analyses were performed using ANOVA F-tests for each variable at alpha = 0.05 whenever the Wilks' Lambda test was significant . If the ANOVA F-test was significant for a given variable, planned comparisons between treatments were done using the LSD method (Fisher protected LSD). When ANOVA F-test was not significant, planned comparisons with the Bonferroni method were performed (LSMEAN option with PROG GLM procedure of SAS. Each of the three color groups was treated individually in the statistical analyses.

## 5.4 Results

As expected, muscle in the light, normal, and dark groups differed in pH values. As reported earlier by Fletcher (1999 a,b) and Fletcher and others (2000), the difference in muscle lightness correlated with initial muscle pH. Before the experiment, muscles in the light group had the lowest pH values, ranging from 5.88 to 5.97, compared to the other two groups. In the normal group muscle pH ranged from 5.99 to 6.15 whereas dark group pH was higher than 6.15. Moreover, the lightness of the three groups did not change significantly during frozen storage and remained the same when samples were defrosted just prior to experimentation. Since the three color groups responded differently to the simulation conditions results will be presented separately for each group.

### Significance of plant and replication

Multivariate analysis of variance showed significant ($P < 0.05$) effect of all tested treatment combinations based on the Wilks' Lambda (data not shown). Therefore,

further analyses, using ANOVA and F statistics, were performed for each independent variable.

The results of the combined ANOVA of raw and cooked meat for replication, plant, and contrasts' effects in pH, ORP, PMD, nitrosopigment, and total pigments measurements are presented in Tables 5.1, 5.2, and 5.3 for the normal, light, and dark group, respectively. There was no significant effect ($P > 0.05$) of replication per plant when all independent variables were tested using a 'replication within plant' as a divisor except for the dark group in replication one for PMD variable. Also, the plant effect was not significant ($P > 0.05$) when tested using residual error as the divisor with the exception for total pigment content of the dark group. Therefore, in subsequent analyses, the data were pooled across plants and replications.

**Normal muscle group with lightness 47 < L* < 50**

Simulation of the pink defect was achieved in nine of the sixteen treatment combinations. An addition of 1 ppm of sodium nitrite produced significant pinking of cooked meat. A combination of sodium chloride, tripolyphosphate, and erythorbate produced pinking without addition of sodium nitrite. There was a significant effect of sodium chloride, tripolyphosphate, and their combinations on increased pH in cooked meat (Figure 5.1-A). However, not all the samples having the highest pH values (6.390–6.414) in this group showed a pink defect. On the other hand, not all pink samples' pH values were significantly different from the control sample's pH of about 6.213. Although the same pH levels occurred for samples injected with sodium chloride, tripolyphosphate and nitrite (6.406) and those injected with sodium chloride and tripolyphosphate (6.414), only the samples containing nitrite showed pinking. These results confirm that pH alone probably cannot cause pinking in cooked meat. Sodium erythorbate affected oxidation-reduction potential changes

Table 5.1: Analysis of variance table (F-statistic; P-probabilities) of pH, oxidation-reduction potential (ORP), percent myoglobin denatured (PMD), nitrosopigment, and total pigment (ppm hematin) for replication (within each plant), plant, and treatment contrasts' effect in the normal group of cooked samples

| Source | Statistic | pH | ORP | PMD | Nitroso pigments | Total pigment |
|---|---|---|---|---|---|---|
| Replication in: | | | | | | |
| Plant 1 | F | 0.47 | 0.43 | 0.10 | 0.00 | 9.34 |
| | P | 0.5635 | 0.5813 | 0.7792 | 0.9581 | 0.0925 |
| Plant 2 | F | 1.96 | 0.60 | 0.10 | 0.01 | 1.43 |
| | P | 0.2963 | 0.5188 | 0.7818 | 0.9280 | 0.3545 |
| Plant | F | 0.47 | 0.09 | 1.33 | 2.84 | 0.01 |
| | P | 0.5630 | 0.7927 | 0.3682 | 0.2340 | 0.9467 |
| Treatments[1] | | | | | | |
| S | F | 293.03 | 149.83 | 11.49 | 1.12 | 4.86 |
| | P | 0.0001 | 0.0001 | 0.0015 | 0.2952 | 0.0327 |
| P | F | 900.01 | 12.58 | 30.05 | 180.19 | 2.18 |
| | P | 0.0001 | 0.0009 | 0.0001 | 0.0001 | 0.1465 |
| SP | F | 72.16 | 3.99 | 2.40 | 138.46 | 0.57 |
| | P | 0.0001 | 0.0520 | 0.1282 | 0.0001 | 0.4548 |
| E | F | 0.15 | 1235.09 | 107.00 | 47.10 | 1.52 |
| | P | 0.6988 | 0.0001 | 0.0001 | 0.0001 | 0.2244 |
| SE | F | 4.97 | 0.62 | 14.87 | 1.15 | 4.16 |
| | P | 0.0308 | 0.4339 | 0.0004 | 0.2900 | 0.0473 |
| PE | F | 1.75 | 25.16 | 14.57 | 13.58 | 6.29 |
| | P | 0.1924 | 0.0001 | 0.0004 | 0.0006 | 0.0158 |
| SPE | F | 39.35 | 2.09 | 14.30 | 24.29 | 0.29 |
| | P | 0.0001 | 0.1548 | 0.0005 | 0.0001 | 0.5909 |
| N | F | 0.00 | 0.53 | 0.02 | 4110.51 | 6.11 |
| | P | 0.9795 | 0.4692 | 0.8929 | 0.0001 | 0.0173 |
| SN | F | 0.16 | 1.06 | 21.22 | 0.18 | 0.45 |
| | P | 0.6907 | 0.3087 | 0.0001 | 0.6766 | 0.5067 |
| PN | F | 3.28 | 10.28 | 10.72 | 82.82 | 1.75 |
| | P | 0.0767 | 0.0025 | 0.0020 | 0.0001 | 0.1928 |
| SPN | F | 2.32 | 1.51 | 31.18 | 120.86 | 1.54 |
| | P | 0.1345 | 0.2258 | 0.0001 | 0.0001 | 0.2205 |
| EN | F | 18.34 | 1.03 | 19.25 | 13.07 | 3.27 |
| | P | 0.0001 | 0.3156 | 0.0001 | 0.0008 | 0.0771 |
| SEN | F | 0.14 | 0.88 | 3.05 | 0.24 | 0.94 |
| | P | 0.7095 | 0.3543 | 0.0875 | 0.6297 | 0.3377 |
| PEN | F | 0.47 | 2.83 | 12.06 | 3.31 | 2.01 |
| | P | 0.4970 | 0.0995 | 0.0012 | 0.0756 | 0.1630 |
| SPEN | F | 0.35 | 17.54 | 34.71 | 29.28 | 0.22 |
| | P | 0.5561 | 0.0001 | 0.0001 | 0.0001 | 0.6394 |

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

Table 5.2: Analysis of variance table (F-statistic; P-probabilities) of pH, oxidation-reduction potential (ORP), percent myoglobin denatured (PMD), nitrosopigment, and total pigment (ppm hematin) for replication (within each plant), plant, and treatment contrasts' effect in the light group of cooked samples

| Source | Statistic | pH | ORP | PMD | Nitroso pigments | Total pigment |
|---|---|---|---|---|---|---|
| Replication in: | | | | | | |
| Plant 1 | F | 8.30 | 1.41 | 0.22 | 0.27 | 3.02 |
| | P | 0.1023 | 0.3572 | 0.6831 | 0.6561 | 0.2244 |
| Plant 2 | F | 13.54 | 3.31 | 0.10 | 0.42 | 1.19 |
| | P | 0.0666 | 0.2105 | 0.7822 | 0.5834 | 0.3896 |
| Plant | F | 0.02 | 0.10 | 0.79 | 0.83 | 0.01 |
| | P | 0.8982 | 0.7814 | 0.4674 | 0.4589 | 0.9140 |
| Treatments[1] | | | | | | |
| S | F | 3.64 | 16.02 | 69.97 | 0.22 | 2.03 |
| | P | 0.0627 | 0.0002 | 0.0001 | 0.6438 | 0.1609 |
| P | F | 148.14 | 136.04 | 95.17 | 2.06 | 0.01 |
| | P | 0.0001 | 0.0001 | 0.0001 | 0.1579 | 0.9096 |
| SP | F | 0.37 | 14.39 | 80.27 | 0.90 | 1.18 |
| | P | 0.5485 | 0.0004 | 0.0001 | 0.3472 | 0.2825 |
| E | F | 0.08 | 196.84 | 48.90 | 16.21 | 25.10 |
| | P | 0.7826 | 0.0001 | 0.0001 | 0.0002 | 0.0001 |
| SE | F | 0.05 | 4.49 | 0.15 | 1.03 | 31.83 |
| | P | 0.8231 | 0.0397 | 0.7036 | 0.3164 | 0.0001 |
| PE | F | 0.01 | 86.78 | 73.50 | 21.13 | 11.32 |
| | P | 0.9124 | 0.0001 | 0.0001 | 0.0001 | 0.0016 |
| SPE | F | 0.03 | 0.00 | 26.45 | 0.05 | 24.06 |
| | P | 0.8648 | 0.9830 | 0.0001 | 0.8173 | 0.0001 |
| N | F | 1.51 | 4.25 | 5.67 | 689.18 | 40.89 |
| | P | 0.2260 | 0.0450 | 0.0216 | 0.0001 | 0.0001 |
| SN | F | 4.56 | 2.35 | 0.44 | 0.89 | 1.18 |
| | P | 0.0381 | 0.1325 | 0.5084 | 0.3518 | 0.2831 |
| PN | F | 1.52 | 6.72 | 27.14 | 3.43 | 13.58 |
| | P | 0.2242 | 0.0128 | 0.0001 | 0.0706 | 0.0006 |
| SPN | F | 0.35 | 1.14 | 0.04 | 4.60 | 19.44 |
| | P | 0.5595 | 0.2922 | 0.8379 | 0.0375 | 0.0001 |
| EN | F | 1.13 | 6.23 | 2.49 | 11.86 | 0.37 |
| | P | 0.2932 | 0.0163 | 0.1214 | 0.0013 | 0.5469 |
| SEN | F | 0.01 | 0.62 | 6.59 | 1.99 | 17.12 |
| | P | 0.9399 | 0.4346 | 0.0136 | 0.1654 | 0.0002 |
| PEN | F | 0.01 | 7.26 | 0.97 | 15.01 | 3.07 |
| | P | 0.9104 | 0.0099 | 0.3290 | 0.0003 | 0.0865 |
| SPEN | F | 0.17 | 0.64 | 0.61 | 0.30 | 1.18 |
| | P | 0.6858 | 0.4279 | 0.4374 | 0.5865 | 0.2833 |

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

Table 5.3: Analysis of variance table (F-statistic; P-probabilities) of pH, oxidation-reduction potential (ORP), percent myoglobin denatured (PMD), nitrosopigment, and total pigment (ppm hematin) for replication (within each plant), plant, and treatment contrasts' effect in the dark group of cooked samples

| Source | Statistic | pH | ORP | PMD | Nitroso pigments | Total pigment |
|---|---|---|---|---|---|---|
| Replication in: | | | | | | |
| Plant 1 | F | 3.86 | 1.32 | 25.45 | 0.01 | 0.09 |
| | P | 0.1885 | 0.3692 | 0.0371 | 0.9266 | 0.7947 |
| Plant 2 | F | 0.34 | 0.06 | 2.66 | 0.82 | 0.00 |
| | P | 0.6208 | 0.8282 | 0.2443 | 0.4603 | 0.9940 |
| Plant | F | 0.48 | 0.25 | 0.02 | 0.38 | 39.00 |
| | P | 0.5608 | 0.6669 | 0.9031 | 0.5988 | 0.0247 |
| Treatments[1] | | | | | | |
| S | F | 0.47 | 56.88 | 0.20 | 77.13 | 8.42 |
| | P | 0.4985 | 0.0001 | 0.6568 | 0.0001 | 0.0057 |
| P | F | 53.80 | 7.70 | 26.84 | 85.01 | 5.71 |
| | P | 0.0001 | 0.0080 | 0.0001 | 0.0001 | 0.0212 |
| SP | F | 6.47 | 53.54 | 19.24 | 8.99 | 4.92 |
| | P | 0.0144 | 0.0001 | 0.0001 | 0.0044 | 0.0317 |
| E | F | 0.05 | 430.39 | 0.13 | 120.30 | 3.02 |
| | P | 0.8181 | 0.0001 | 0.7206 | 0.0001 | 0.0890 |
| SE | F | 3.63 | 36.36 | 12.83 | 0.59 | 1.80 |
| | P | 0.0631 | 0.0001 | 0.0008 | 0.4475 | 0.1861 |
| PE | F | 1.63 | 0.06 | 5.99 | 43.76 | 0.09 |
| | P | 0.2085 | 0.8046 | 0.0184 | 0.0001 | 0.7594 |
| SPE | F | 1.12 | 7.00 | 6.57 | 27.28 | 0.09 |
| | P | 0.2956 | 0.0112 | 0.0138 | 0.0001 | 0.7605 |
| N | F | 8.97 | 0.18 | 8.70 | 3241.60 | 0.46 |
| | P | 0.0044 | 0.6711 | 0.0050 | 0.0001 | 0.5023 |
| SN | F | 0.03 | 6.33 | 0.12 | 43.69 | 0.20 |
| | P | 0.8567 | 0.0155 | 0.7307 | 0.0001 | 0.6589 |
| PN | F | 8.67 | 0.85 | 35.72 | 54.65 | 7.02 |
| | P | 0.0051 | 0.3609 | 0.0001 | 0.0001 | 0.0111 |
| SPN | F | 2.07 | 5.39 | 3.02 | 11.76 | 2.17 |
| | P | 0.1571 | 0.0248 | 0.0888 | 0.0013 | 0.1475 |
| EN | F | 2.90 | 2.56 | 2.12 | 108.18 | 0.05 |
| | P | 0.0954 | 0.1163 | 0.1522 | 0.0001 | 0.8238 |
| SEN | F | 0.41 | 14.71 | 13.13 | 2.42 | 0.02 |
| | P | 0.5251 | 0.0004 | 0.0007 | 0.1265 | 0.8807 |
| PEN | F | 3.10 | 5.06 | 3.86 | 19.28 | 0.76 |
| | P | 0.0852 | 0.0294 | 0.0556 | 0.0001 | 0.3885 |
| SPEN | F | 0.45 | 6.19 | 3.68 | 36.18 | 0.64 |
| | P | 0.5064 | 0.0166 | 0.0614 | 0.0001 | 0.4297 |

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite

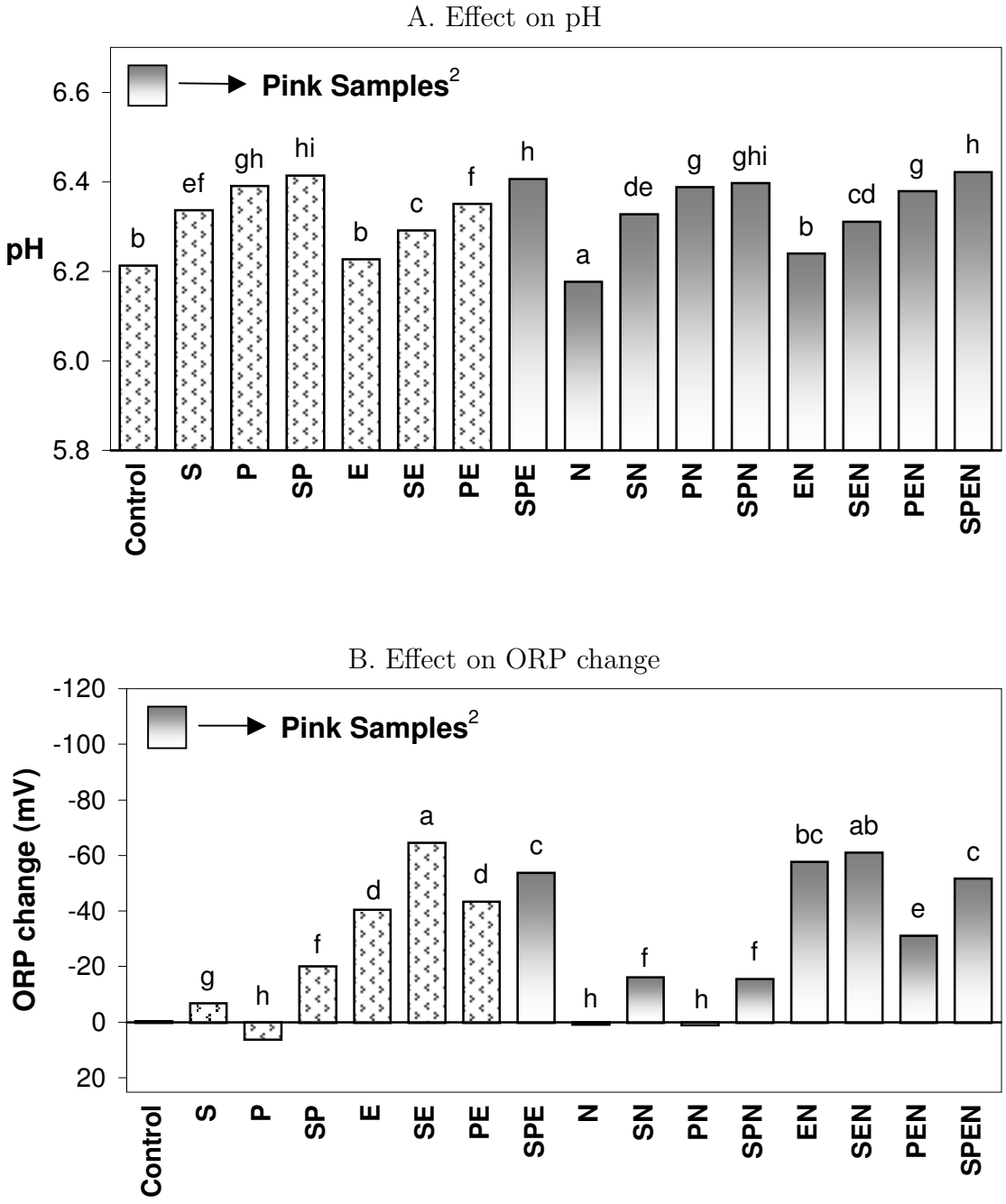
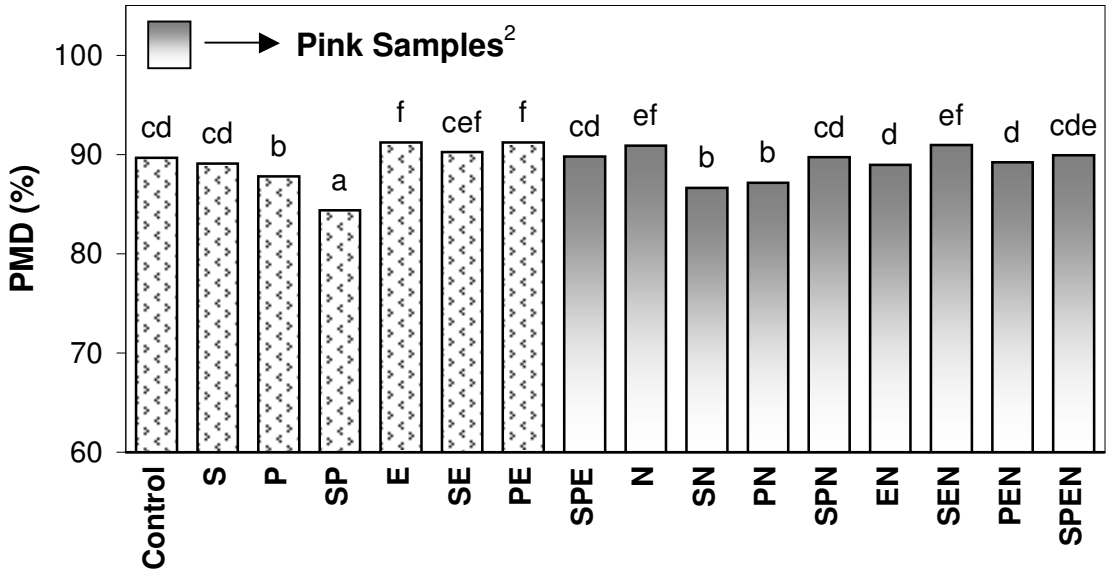



Figure 5.1: Effect of treatment combinations[1] on different variables in cooked chicken breast fillets from the normal group

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-i}$Bars with no common letters differ significantly ($P < 0.05$)

C. Effect on percent myoglobin denatured (PMD)




D. Effect on nitrosopigment

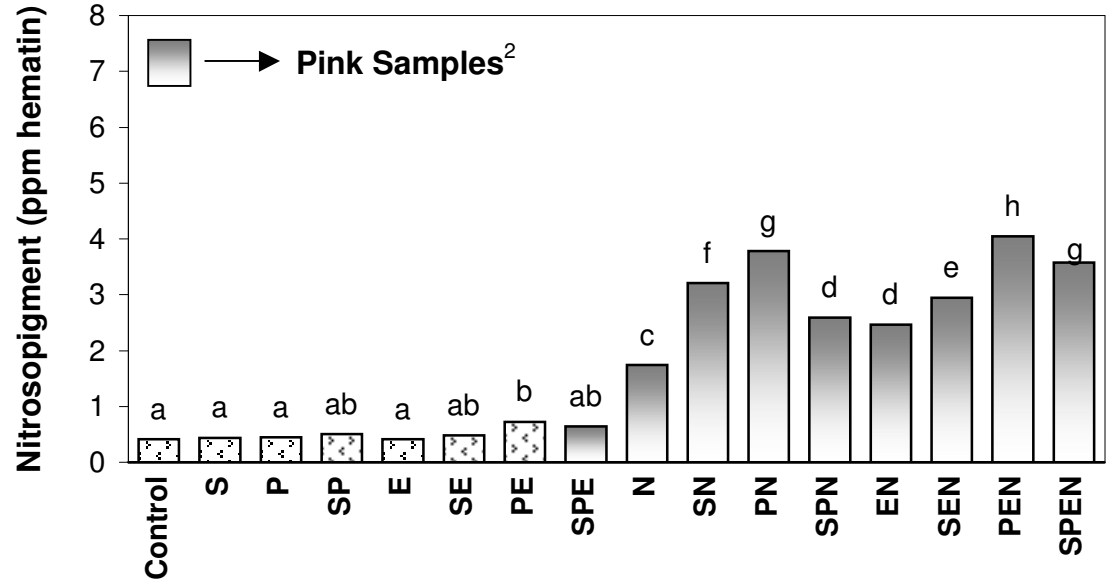



Figure 5.1: Cont.

E. Effect on total pigment

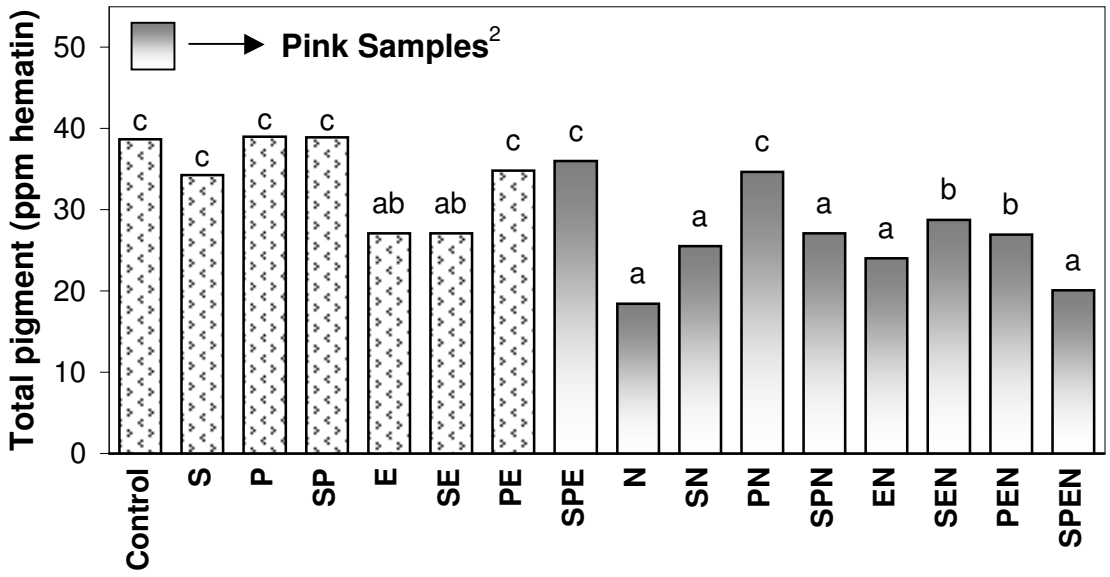



Figure 5.1: Cont.

in cooked samples (Figure 5.1-B). The greatest change in ORP values compared to the control was found both in samples injected with a combination of sodium chloride and erythorbate (−64.15) and in those injected with a combination of sodium chloride, erythorbate and nitrite (−61.22). Only the latter sample was pink. Among pink samples containing nitrite those having sodium erythorbate showed significant increase in reducing conditions. Samples injected with sodium nitrite alone or with nitrite and tripolyphosphate showed an oxidation-reduction potential similar to that of the control cooked samples. This finding may indicate that a high negative oxidation-reduction potential (ORP $< -120$mV) as one of in situ conditions in cooked meat is favorable for pinking without the presence of sodium nitrite. The presence of sodium tripolyphosphate with sodium chloride did decrease the percentage of myoglobin denatured but the effect was slightly diminished when those ingredients were present in combination with nitrite and erythorbate (Figure 5.1-C).

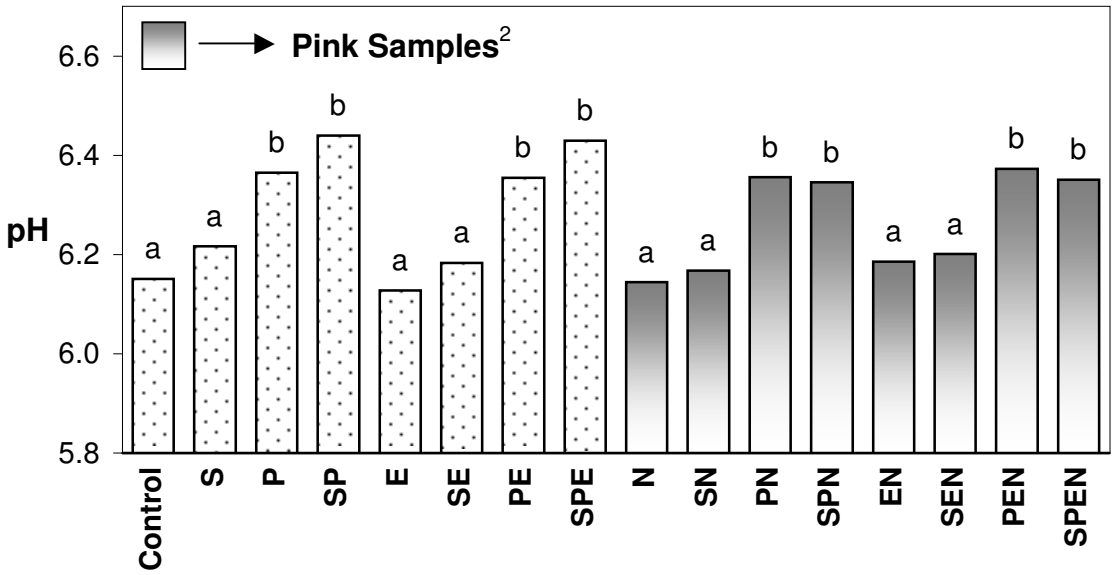
Among pink samples only those containing sodium chloride or tripolyphosphate had a significantly lower percentage of myoglobin denatured (PMD) (86.7% and 87.1%). The fact that some of the pink samples showed PMD to be not significantly different from the control may indicate that undenatured pigments are not a deciding factor in pinking of chicken breast meat. Undoubtedly, the presence of just 1 ppm of sodium nitrite in the injection solution did effect pinking. As shown on Figure 5.1-D, samples containing nitrite showed significantly higher nitrosyl hemochrome concentration compared to the control sample, and these samples exhibited pinking. The combination of sodium chloride, tripolyphosphate, and erythorbate did not show higher nitrosyl hemochrome concentration than the control, indicating that simulation was possible without the addition of sodium nitrite as a pinking agent. Total pigment, expressed as the ppm of hematin in meat, showed marked variation among pink and non-pink samples with pigment values ranging from 20 ppm to 38 ppm (Figure 5.1-E). In absence of nitrite total pigments values were generally higher than the treatments in presence of nitrite. Also in absence of nitrite total pigment values were not affected due to treatment with the exception of two treatments — erythorbate and sodium chloride plus erythorbate.

**Light muscle group with lightness L* > 50**

The only pink samples in this group were those containing sodium nitrite as one of the ingredients in the injection solutions. This group was prone to pH changes caused by sodium tripolyphosphate (Figure 5.2-A). The highest pH value found in this group was 6.443. However, the pH increase in this group did not appear to effect pinking. Among all the pink samples, those injected with sodium tripolyphosphate had a significantly higher pH than the control, and the rest of the pink group had the same pH as the control sample (6.151). Induced changes in oxidation-reduction potential were

affected by the presence of both sodium erythorbate and tripolyphosphate. Increased level of reducing conditions represented by more negative ORP change values did not correspond to pinking (Figure 5.2-B). Samples injected with sodium nitrite only or a combination of sodium chloride and nitrite showed significantly smaller ORP changes compared the rest of the samples with pink defect.The ORP changes of the other pink samples ranged from −55.27 to −81.58 and were not statistically different from samples with no pink defect. The greatest ORP change in the light group, −99.18, for a combination of sodium chloride, tripolyphosphate, and erythorbate did not produce a pink defect. The percent of myoglobin denatured exhibited considerable variability, even in the samples with pink defect (Figure 5.2-C). There was a significant effect of sodium tripolyphosphate and sodium chloride treatment on myoglobin denaturation. However those changes did not correlate with pinking. The lowest PMD in the pink samples, 80.02% was found after injection with a combination of sodium chloride, tripolyphosphate and nitrite. However, the PMD values of samples subjected to sodium chloride and tripolyphosphate — with and without nitrite — were not significant. The samples with the simulated pink defect in the light group had a significantly higher concentration of nitrosyl hemochrome compared to the rest of the light group (Figure 5.2-D). When sodium nitrite was accompanied by sodium tripolyphosphate or erythorbate, further increase in nitrosopigment concentration was detected. It was also noted that no significant effect on nitrosopigment due to treatments was found when sodium nitrite was not included. Figure 5.2-E illustrates significantly lower concentration of hematin in pink samples (between 16.68 and 22.85) compared to the rest of the light group (from 26.32 to 35.89), this is attributed to a high concentration of nitrosopigment (Figure 5.2-D).

A. Effect on pH




B. Effect on ORP change

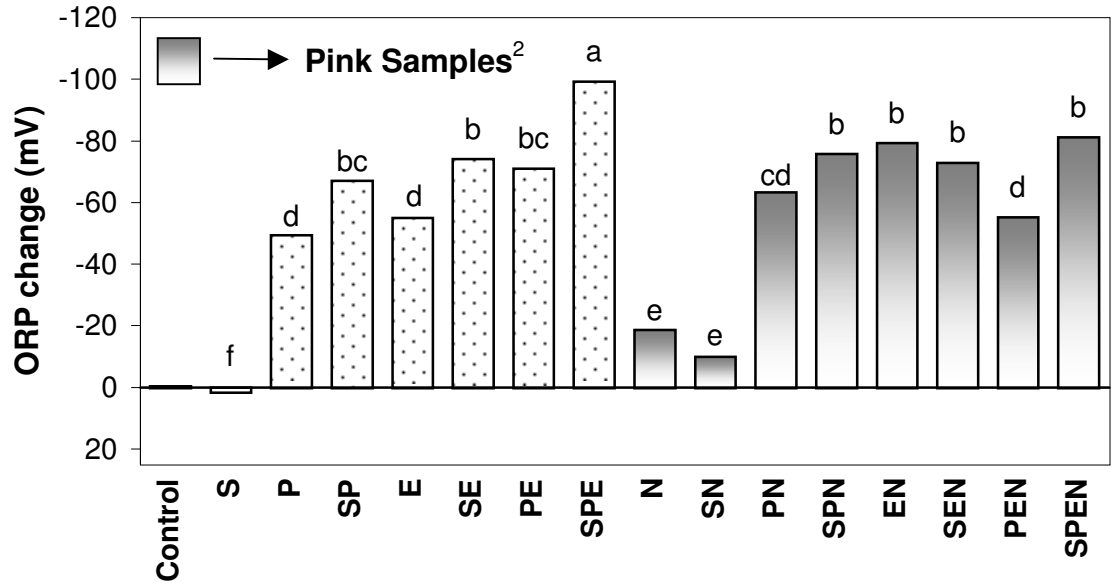
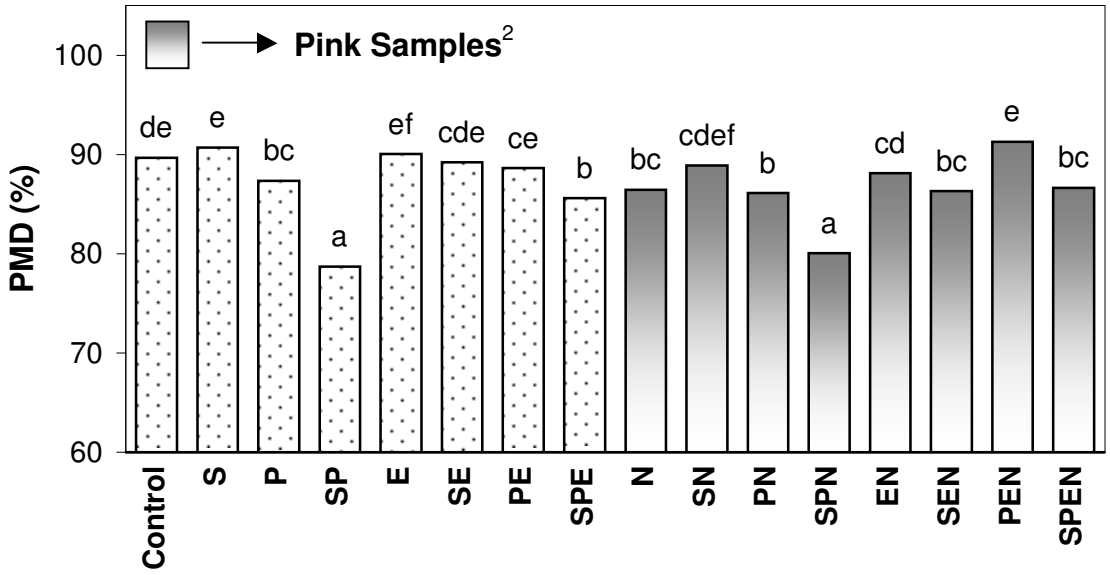



Figure 5.2: Effect of treatment combinations[1] on different variables in cooked chicken breast fillets from the light group

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-f}$Bars with no common letters differ significantly ($P < 0.05$)

C. Effect on percent myoglobin denatured (PMD)




D. Effect on nitrosopigment

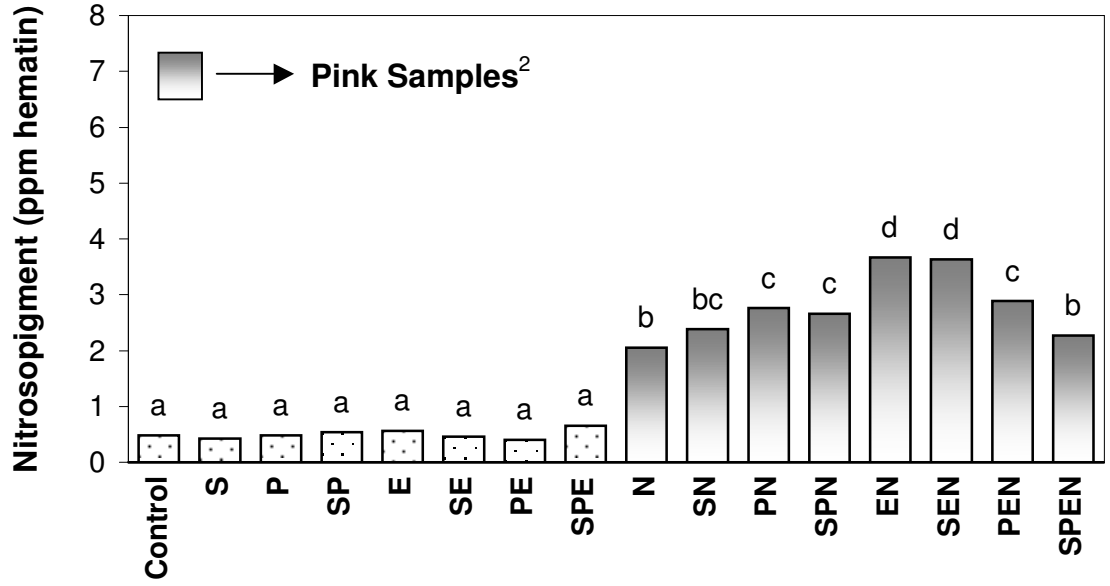



Figure 5.2: Cont.

E. Effect on total pigment

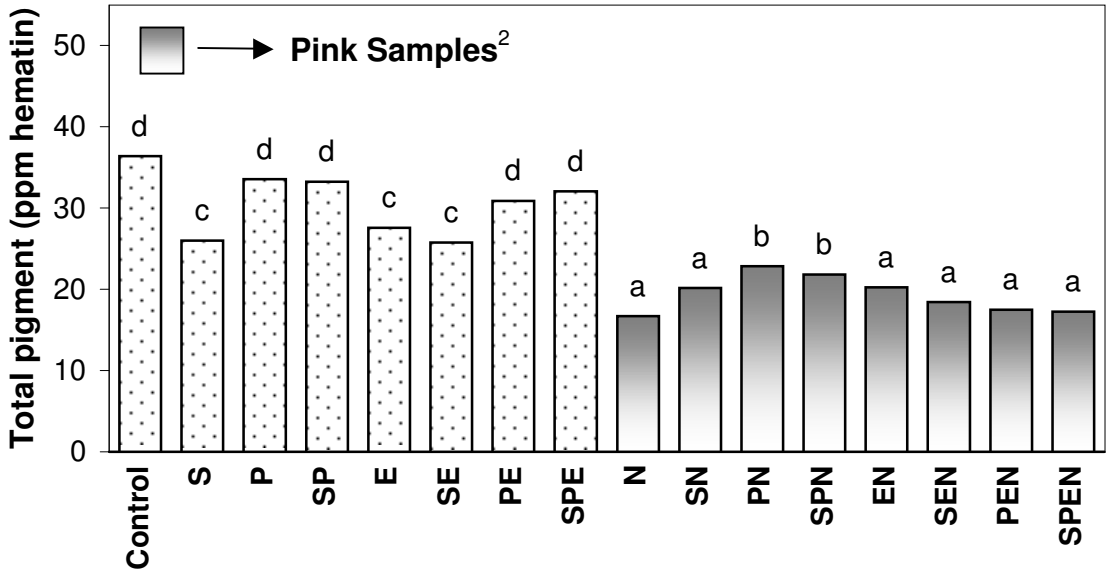



Figure 5.2: Cont.

**Dark muscle group with lightness L* < 47**

Simulation of the pink defect in this group was produced in ten of the sixteen treatment combinations. Pink samples included all samples containing sodium nitrite and two other samples injected with a combination of sodium chloride with tripolyphosphate and a combination of sodium chloride, tripolyphosphate and erythorbate. With the high pH (6.379) of cooked meat of the control samples, the effect of tripolyphosphate or sodium chloride with tripolyphosphate in the dark group was not as large as in the normal and dark groups (Figure 5.3-A). Among the pink samples, the highest pH, 6.499 – 6.502, was found in those injected with a combination of sodium chloride, tripolyphosphate, and erythorbate, or a combination of sodium chloride, tripolyphosphates and nitrite, or a combination of all four ingredients. As in the normal and light groups, the dark group's high pH did not always

translate into the pink defect. Even with the significant effect of sodium erythorbate on ORP changes in this group, the effect was not the same as in the other two muscle groups (Figure 5.3-B).ORP change in the pink samples was in the range of only −5.17 to −29.32, indicating that the initial oxidation-reduction potential of the control sample was already so low that the applied treatment combinations did not cause a further increase of reducing conditions. The dark group showed the most consistent response in myoglobin denaturation to the induced conditions (Figure 5.3-C). Among the pink samples in the dark group, a combination of sodium chloride, tripolyphosphate and erythorbate showed significantly lower PMD, 86.54%, compared to the rest of the pink samples. The percentage of myoglobin denatured in the pink samples was significantly lower compared to the control and was always below 90%. For nitrosopigment, a highly significant effect for sodium nitrite was found for the pink samples (Figure 5.3-D). This effect was indicated by two- and three-fold increases in nitrosopigment concentration when sodium nitrite was present in combinations with other ingredients when compared to the samples injected with sodium nitrite alone. The pink samples that did not contain sodium nitrite had nitrosyl hemochrome concentration similar to that of the rest of the non-pink samples (0.616–0.923). Samples with the pink defect containing sodium nitrite as one of the ingredients showed lower concentration of hematin when compared to the rest of the group (Figure 5.3-E). The other pink samples not affected by nitrite did not show significant differences in total pigment content compared to the non-pink samples.

## 5.5 Discussion

A relation between the initial muscle properties and the lightness of the muscle was previously reported by Fletcher (1999 a,b,c) and Allen and others (1998). Similarly,

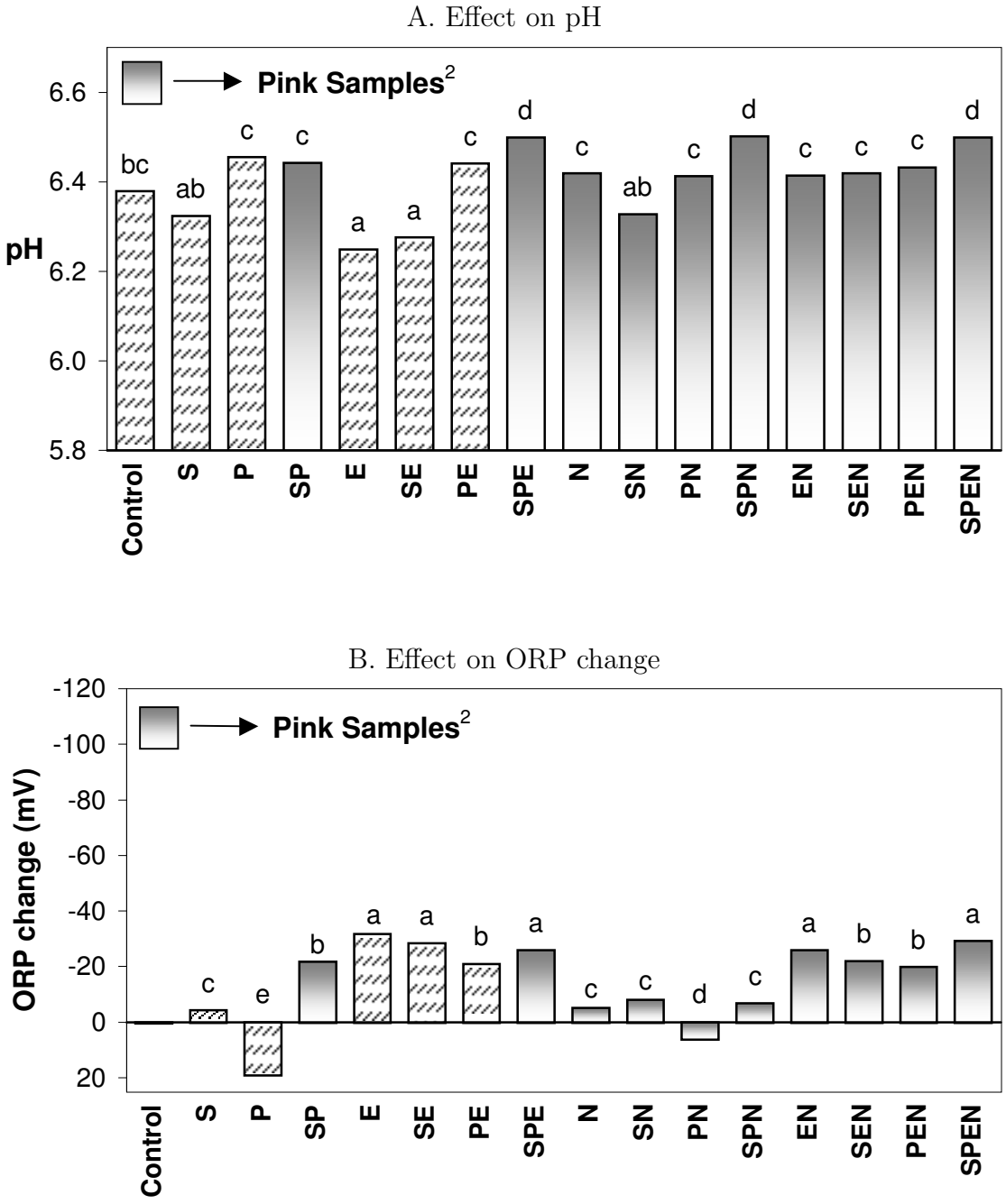
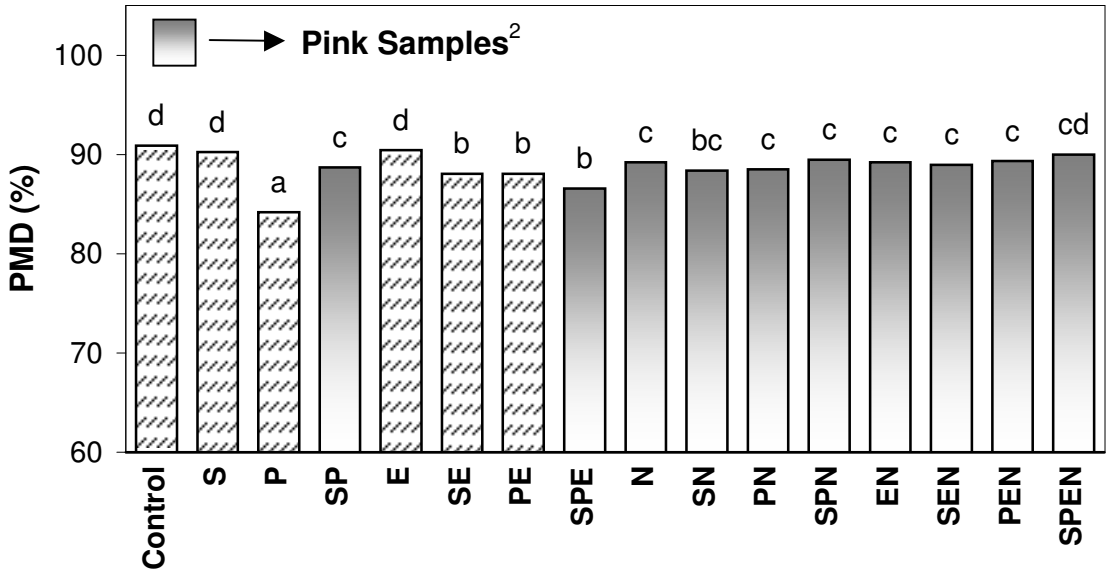



Figure 5.3: Effect of treatment combinations[1] on different variables in cooked chicken breast fillets from the light group

[1]S-Sodium chloride; P-Sodium tripolyphosphate; E-Sodium erythorbate; N-Sodium nitrite
[2]Samples exhibited pinking after cooking
$^{a-f}$Bars with no common letters differ significantly ($P < 0.05$)

C. Effect on percent myoglobin denatured (PMD)




D. Effect on nitrosopigment

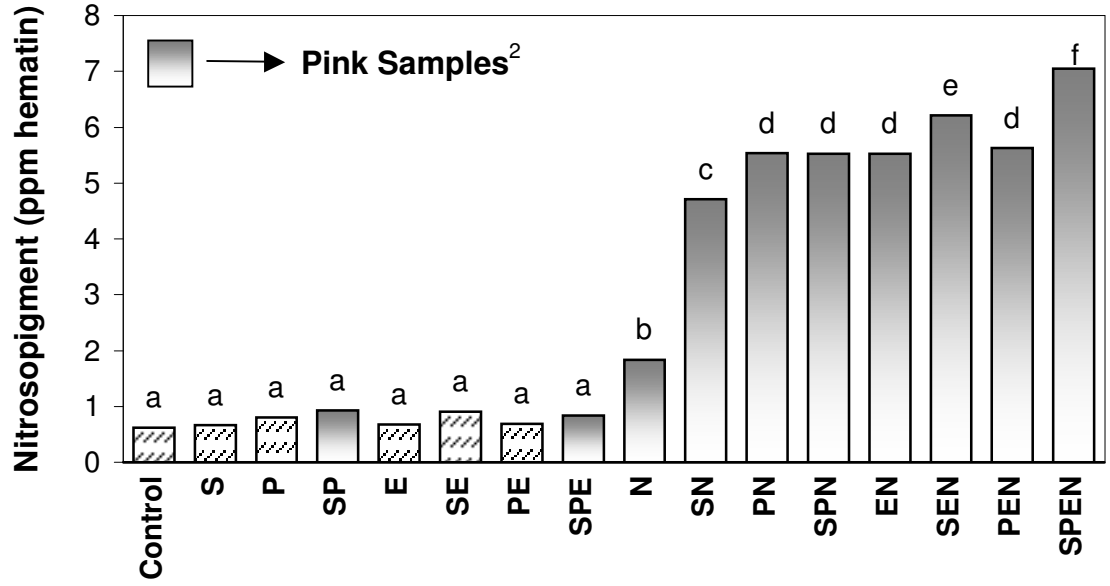



Figure 5.3: Cont.

E. Effect on total pigment

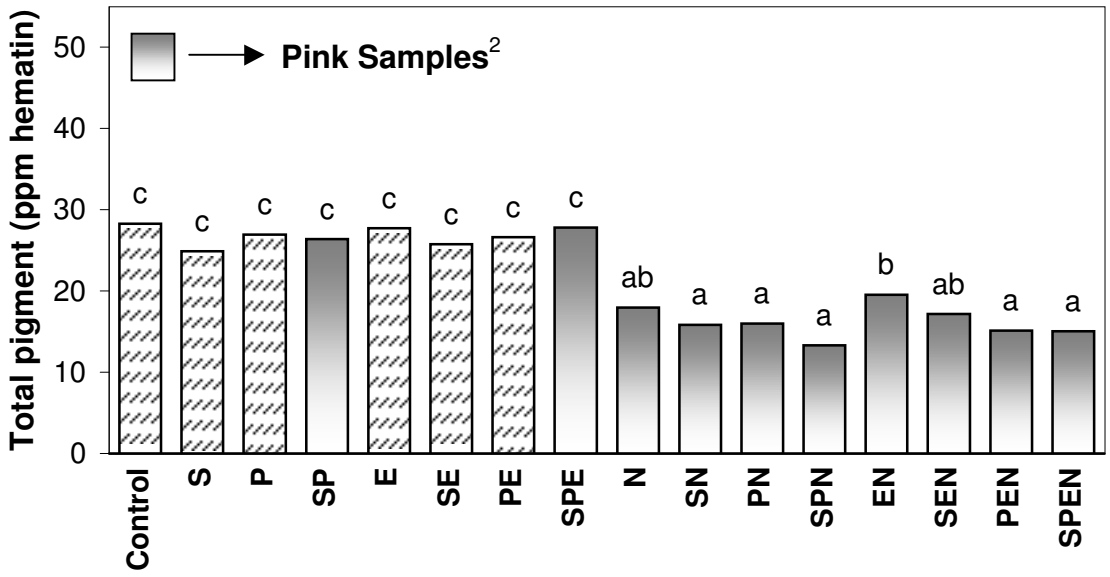



Figure 5.3: Cont.

our study showed differences in the normal, light, and dark groups, not only in pH and oxidation-reduction potential, but also in response to the applied experimental treatments. Sodium nitrite has been studied as a pinking agent by a number of authors (Nash and others 1985; Ahn and Maurer 1987, 1989a; Heaton and others 2000). Heaton and others (2000) demonstrated that as little as 1 ppm of nitrite can cause pinking under certain conditions in chicken and turkey meat. Our results confirmed that regardless of the muscle color group pinking occurs when 1 ppm or more of sodium nitrite is introduced into the meat before cooking. Cornforth and others (1986) showed the importance of reducing conditions on the formation of pink hemochromes in cooked white meat. They indicated that an oxidation-reduction potential (ORP) of −550 mV or less is preferable for the iron to be in a ferrous state. In our study, however, the samples had different initial ORP values. The best indicator of the ORP changes in the direction of the reducing conditions was the

ORP difference between treated samples and controls. Also, we found that pinking in samples containing sodium nitrite was not related to the reducing conditions. Nam and Ahn (2002) reported lower ORP values as a potential for pinking in irradiated turkey breast meat; their reported ORP values were in the range of our study. Van Laack and others (2000) found a correlation between pH and lightness of chicken breast muscle. In addition, Young and others (1996b) showed that any effect of tripolyphosphate on increasing meat pH depends on the initial lightness and pH of the muscles. The three preselected (based on lightness) muscle groups in our research responded differently to treatment factors, and those responses were affected by initial muscle pH. Furthermore, to what extent myoglobin denatures during cooking was greatly affected by pH as described earlier by Janky and Froning (1973) and Fletcher (1999b). Although, undenatured pigments may be responsible for pinking in cooked meat under particular conditions, they are not the only factor. Ahn and Maurer (1990b) indicated that a pH $>$ 6.4 was favorable for the heme-complex-forming reactions of pigments with ligands. Especially when sodium nitrite concentration was lower than 1 ppm, the in situ conditions such as high pH and low ORP were the strongest factors affecting the reactivity of pigments.

As reported earlier (Holownia and others 2002b), induced raw meat conditions also differed among the three color groups. In that we showed nitrosopigment content in raw meat to be a good predictor of pinking for all three color groups. It was further confirmed, regardless of the muscle color group, that nitrosopigment concentrations prior to and after cooking were similar. Cooked meat pH of the normal and dark groups seem to be more related to pinking compared to that of raw meat conditions for the same two groups studied previously (Holownia and others 2002b). The effect of sodium tripolyphosphate on increased pH was similar in raw and cooked meat from the normal and light groups. Initial high raw meat pH values in the dark group, due to the response to tripolyphosphate diminished after cooking. Neither cooked

nor raw meat pH of the light muscle group was a good indicator of pinking. In this group only nitrosopigment content was a deciding factor. The addition of sodium erythorbate produced similar reducing conditions in raw meat and cooked meat for the normal and light color groups. On the other hand, the dark group reducing conditions in cooked meat did not correspond to the conditions found in the raw meat. Pink samples from normal group injected with sodium chloride, tripolyphosphate and erythorbate showed high reducing conditions in both raw and cooked meat. We previously reported that in situ conditions of raw meat as induced by different ingredients as well as the initial lightness of chicken breast muscles affected development of pinking (Holownia and others 2002b). Similarly, the induced conditions found in cooked meat in this study were also affected by the initial lightness of raw muscles.

## 5.6 Conclusions

The simulation, using four factors, sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite reproduced the pink color defect in cooked chicken breast meat. The simulation was achieved both with and without the addition of 1 ppm of sodium nitrite. A combination of different induced in situ conditions, rather than a single in situ condition, was responsible for the pink defect. Induced high pH (>6.2) values and reducing conditions (ORP $< -120$mV) were responsible for the simulated pink defect in the absence of sodium nitrite. With no significant effect of either replications nor plants we conclude that the simulation of the pink defect could be used as a tool in further research to investigate procedures to reduce or eliminate pinking.

**Acknowledgments**

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# Chapter 6

# Summary and Conclusions

*To love what you do and feel that it matters*
*—how could anything be more fun?*
—Katharine Graham

The pink color defect in cooked poultry white meat is associated by consumers with being undercooked and unsafe to eat. Even though the USDA Food Safety and Inspection Service regulation requires that heat-processed poultry products sold as ready-to-eat must be cooked to 71.1 °C, the pink defect may be responsible for more than $10,000,000 of annual losses due to rejection, rework, or condemnation. More than 1.25 billion broilers are slaughtered and processed into food products each year in Georgia alone. With such huge numbers, even a small proportion of defect can translate into a very substantial economic loss for the poultry industry.

Interactions of factors and mechanisms associated with the pink defect are complex, as a result a large systematic study was initiated to understand the occurrence of pinking. Simulation of the pink defect was used to serve as a research tool to examine the induced raw and cooked meat conditions associated with this defect. The simulation was achieved by modifying the in situ conditions of the meat using ingredients that are commonly employed by the industry. The in situ changes were induced using four factors: sodium chloride (1g/100g meat), sodium tripolyphosphate (0.5g/100g meat), sodium erythorbate (0.0546g/100g), and sodium nitrite (1 ppm) at two levels: present and not present. Samples of the three color groups (light, normal, and dark) of boneless, skinless, chicken breast muscles based on CIE L* (lightness) were selected for the experiment. The selection was important because the lightness of raw muscles indicated different initial endogenous conditions of meat and different response of the samples to the experimental treatments. Muscles in all treatments were subjected to individual injections (12% of meat weight basis), followed by a tumbling process, cooking and chilling. Induced color and in situ changes

in samples before and after cooking were analyzed using visual objective appraisal, reflectance spectrophotometry, analyses of pH, oxidation-reduction potential, and pigments.

The following conclusions are drawn from the study:

- It was possible to simulate a pink defect in cooked chicken white meat using the in situ conditions of raw meat induced by combinations of sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite.

- Simulation of the pink defect was achieved both with and without sodium nitrite. Simulation was most effective in the dark group, followed by the normal and light groups. The initial lightness (CIE L*) of the raw muscles was found to be the most critical condition for the occurrence of pinking.

- Establishing of a subjective pink threshold was an important step towards setting a cutoff point for judging the existence of pinking during simulation. Different levels of sodium nitrite addition as a pinking agent resulted in the establishment of the pink color threshold at CIE a* value equal to 3.8 (assuming that CIE color values of L* and b* of the samples ranged from 79 to 84 and from 10 to 16, respectively).

- The logistic regression model was effective in relating the induced in situ conditions of raw meat to the occurrence of pinking in cooked meat while considering the natural variation in color of chicken breast muscles. The logistic models for each of the light, normal, and dark groups correctly predicted more than 90% of the pinking in cooked samples, thus establishing the usefulness of such models to the poultry processing in assessing the potential of pinking. A robust method was used to test reproducibility of pink defect. The method involved

employment of more than one thousand samples from two very different processing plants to develop the initial models, and then validating the models using independently taken samples.

- Regardless of the initial raw muscle's lightness, pinking occurred when 1 ppm or more of sodium nitrite was either naturally present or introduced into the meat before cooking.

- Common processing ingredients, such as sodium chloride, tripolyphosphates, and sodium erythorbate formed favorable conditions for heme-complex formation through hemochrome combination with naturally present and added nitrites to form pink nitrosyl hemochrome.

- A combination of different induced conditions of raw meat, rather than a single in situ condition, produced simulated pink defect. Higher than 6.0 pH values of raw meat and highly reducing conditions (ORP $< -120$mV) induced during simulation evidently modified susceptibility of meat pigments to denaturation, pigments' chemical state, and ligands reactivity with the heme iron.

- Along with nitrosopigments, reduced globin hemochromes and cytochrome c in the cooked meat contributed the most to occurrence of the pink defect.

A single most important factor responsible for pinking in poultry muscle is presence of nitrates and nitrites associated with various aspects of poultry production and processing. Thus, the poultry industry must ensure that the sources of nitrate and nitrite in their plants are minimized. It is recommended for the poultry processors to ensure that there is not significant variation in time postmortem of the meat that is processed in their plant. Such variation may cause changing muscles' pH and associated lightness of the muscle. High reducing conditions of muscles postmortem in combination with high pH are favorable for developing pinking. Excessive use

of high pH marinades may also increase the occurrence of pink color in the final product.

The continuation of the simulation study will lead to a development of the pink threshold as a guideline for the poultry industry. Also, further investigation of occurrence of chemical changes associated with pinking will aid in developing alternative processing methods to eliminate any pink defect.