Reduction of Pink Color Development in Cooked, Uncured Ground Turkey Breast by the Addition of Dairy Proteins

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(Abstract)

A sporadic pink color development in cooked, uncured turkey products remains a problem within the poultry industry because consumers associate this defect with inadequate cooking. Previous research demonstrated that nonfat dry milk (NFDM) has the ability to reduce pink color. The objective of this research was to determine if other dairy proteins also possess this capability. In particular, sodium caseinate (SC) and whey protein concentrate (WPC) were evaluated and compared to nonfat dry milk and to no dairy protein containing processed turkey.

Pink color development was induced in the poultry products to simulate this defect in products by the addition of nicotinamide to produce nicotinamide hemochrome or sodium nitrite or sodium nitrate to produce nitrosylhemochrome. Prior to protein testing, measurement of these two pigment using reflectance spectrophotometric methods was evaluated. The reflectance ratio of %R at 537 nm divided by %R at 553 nm was able to predict (R^2=0.99) concentrations of nicotinamide up to 2%, the highest level tested. The ratio of %R at 650 nm divided by %R at 570 nm was able to predict nitrite (R^2=0.97) below 20 ppm.

To narrow the possible dairy protein choices, three WPC and two SC dairy proteins, along with nonfat dry milk were evaluated for their ability to inhibit nicotinamide and nitrite induced pink color. Results of this prescreening indicated that variations among the different types of proteins existed in both their abilities to reduce the pink color when pink color generating ligands were intentionally added, and when no ligands were added. Some of the dairy proteins actually increased the redness of the control turkey formulation.

The WPC (Alacen 882, New Zealand Milk Products, North America, Inc, Santa Rosa, CA) and SC (Alanate 180 New Zealand Milk Products, North America, Inc., Santa Rosa, CA) protein products chosen in the prescreening were evaluated with nonfat dry

milk at various levels. A simplex lattice response surface design enabled prediction of these proteins' effects on red color at combinations of up to and including 3.0% added dairy protein. Sodium nitrate did not appear to increase redness of control samples and therefore was not discussed in detail. The WPC and NFDM proteins tested were able to reduce CIE a* values at both 1.5 and 3% and in combination with each other at 1.5% of each protein ($P<0.05$) regardless of ligand treatment. Of these treatments, SC had the least effect on CIE a*. With the exception of SC, the dairy proteins increased product yield ($P<0.05$) in all treatment combinations. Using the response surface prediction ability, other combinations of dairy proteins, not specifically tested in this research, were shown to optimize pink color reduction.

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Chapter 1

Introduction

The poultry industry has identified a sporadic pink color development in cooked, uncured turkey products as both a visual and economic problem. Consumers accustomed to the typical light brown color of turkey breast could mistake pink turkey as being undercooked, and possibly unsafe. Producers of turkey products are sometimes forced to redirect the affected product and discount prices causing economic losses. Most efforts to eliminate the pinking defect have focused on reducing nitrite contamination within the manufacturing process. Another possible solution to the pink color development would be to find an ingredient, such as dairy proteins, that could be added to the turkey breast formulation to prevent the pink color from developing.

Nitrite in uncured turkey products will cause a pink color in cooked products due to the formation of nitrosylhemochrome (NITHEME), a heat stable pink pigment. NITHEME is the typical pigment associated with cured meats where nitrite is intentionally added.

Nitrites can be introduced into products in several manners. The most critical factor, and easiest to solve, would be contamination of nitrites from a cured product coming in contact with the non-cured product. In this case, avoiding direct contact, proper sanitation, and storing products separately should eliminate this problem. Nitrites in feed (Froning et al., 1969) and cooling water (Nash et al., 1985) have also been reported to increase red color in cooked products.

Pink pigment identification in other meat products has led to the identification of other pink pigments in turkey products. Brown and Tapple (1957) determined that denatured hemochrome complexes were responsible for the pink color of cooked and canned tuna. Because nicotinamide is able to react with the heme containing pigments and is found at relatively high concentrations in tuna, nicotinamide hemochrome (NICHEME) was suspected as the pink pigment. Ghorapade and Cornforth (1993) determined that denatured globin hemochrome complexes were the cause of the pink color development in cooked pork roasts. Howe et al. (1982) and Ghorapade and Cornforth (1993) reported that the pink color faded quickly upon exposure to air and light.

Cornforth et al. (1986) attributed the sporadic pink color development in turkey to hemochrome complexes. The authors found that reflectance patterns from commercially pink samples were similar to the reflectance patterns of experimental samples containing nicotinamide. Reducing conditions within the meat block were reported to be important in the formation of the hemochrome-complexes.

Dobson and Cornforth (1992) examined the ability of nonfat dry milk (NFDM) and whey protein concentrate (WPC) to reduce pink color development of stored turkey samples. NFDM was found to reduce redness (Hunter a values). The authors speculated that NFDM's ability to reduce the pink color in turkey could be related to an increase of the oxidation-reduction potential leading to a reduction in the formation of denatured hemochromes.

Schwarz et al. (1997) found NFDM to reduce red color in ground turkey products when sodium nitrite (150 ppm), nicotinamide (1.0%) or no ligand was added. Schwarz et

al. (1998) found similar results in a whole breast product. The authors reported that at least 2% NFDM was necessary for a reduction in CIE a* values in no added ligand and nicotinamide treated samples. In this work, no reduction in CIE a* values was found in nitrite containing samples.

In general, dairy proteins can be incorporated into meat products to improve the water holding capacity, improve texture, and improve color (van den Hoven, 1987). These improvements in product quality are related to specific functional properties that vary among the numerous types of dairy proteins available. Dairy proteins, when added to processed meat, can improve the water holding capacity and fat emulsification (van den Hoven 1987). The use of dairy proteins to stabilize the fat allows the extracted meat proteins to bind more water (Hoogenkamp, 1986). Milk proteins can also be added to processed meats to enhance gel formation upon heating (Hoogenkamp, 1986). Gel formation is an important factor in the texture of the meat products.

The objective of this research was to determine the ability of dairy proteins to reduce pink color in turkey breast products. Because the pink color development in these products is sporadic, pink color generating ligands (nicotinamide and nitrite) were added to the turkey formulations.

The research was divided into three separate, but complimentary projects. The objective of the first project was to further evaluate the validity of the measurements of NICHEME and NITHEME in turkey products in which a greater number of levels and lower levels of nicotinamide and nitrite were incorporated than in previously reported studies. With measurement techniques in place, the second project was designed to prescreen the numerous dairy protein products available from suppliers. In this research,

two sodium caseinates and three WPC protein products were evaluated for their ability to reduce red color. From this research, one of each type of dairy protein was chosen to use in the comprehensive testing. The third project was designed as a response surface design to allow for the prediction of the proteins' abilities to reduce red color over a wide range of combinations of treatments. The use of EDTA was also evaluated for its ability to reduce red color in the presence of both dairy proteins and the pink color generating ligands.

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Chapter 2

Literature Review

2.1 DAIRY PROTEINS

2.1.1 Dairy proteins to reduce pink color development

Dobson and Cornforth (1992) examined the ability of nonfat dry milk (NFDM) and whey protein concentrate (WPC) at 3.0% to reduce pink color development of stored turkey samples. The authors based their research on work showing that NFDM was able to lighten the color of bologna (Rongey and Bratzler, 1966) and that calcium caseinate has a whitening effect on chicken nuggets (van den Hoven, 1987). Dobson and Cornforth (1992) reported that NFDM reduced redness (Hunter a values), but had no effect on the lightness of the samples. In that research, WPC increased the redness to a level that was "visibly pink" upon cutting. The authors speculated that NFDM's ability to reduce the pink color in turkey could be related to an increase of the oxidation-reduction potential leading to a reduction in the formation of denatured hemochromes.

Schwarz et al. (1997) found that NFDM reduced red color in ground turkey products when either sodium nitrite (150 ppm), nicotinamide (1.0%) or no ligand was added. The authors concluded that the reduction in redness by NFDM due to nitrite addition was not visually evident. However, it is possible that the high level of nitrite added overwhelmed the system by binding all of the available pigments. Additionally, a level of 150 ppm, that used by Schwarz et al. (1997), would not be indicative of a contamination event.

In further work, Schwarz et al. (1998) found similar results in a whole breast system. The authors reported that at least 2% NFDM was necessary for a reduction in CIE a* values in no added ligand and nicotinamide treated samples. In this work, no reduction in CIE a* values was found in nitrite containing samples.

2.1.2 Composition and sources of dairy proteins

Hoogenkamp (1989) detailed the components of cow's milk. Milk is mainly composed of water (87.3%), but also contains lactose (4.8%), minerals (0.8%), fat (3.7%), and protein (3.4%). The protein content can be divided into the casein (2.8%) and whey (0.6%).

Casein and whey proteins are classified based on their solubility. Casein proteins, valuable for their use in cheeses and other industrial products, have a low solubility at pH 4 to 5, whereas whey proteins are soluble at the same pH (Hambraeus, 1982). Through the use of lactic acid producing bacteria, or by the addition of acid, casein's low solubility is utilized to coagulate and separate it from the liquid components of milk.

Casein accounts for 76 to 86% of the total protein in raw milk and consists of four gene products including α_{s1}-caseins, α_{s2}-caseins, β-caseins, and κ-caseins (Swaisgood, 1982). Other types of caseins present in milk are the products of post-transnational processing. Such processes as phosphorylation, glycosylation, and limited proteolysis are involved in the formation of other caseins including the γ-caseins which are derived from β-caseins (Swaisgood, 1982). With a high-proline and low sulfuric amino acid content, caseins have a mostly random coil structure with a low helix content (van den Hoven, 1987).

After coagulation of the casein proteins, the liquid portion, or whey, contains many of the minerals and soluble proteins. The soluble proteins, known as whey proteins, have been historically dumped into the waste system or used as fertilizer (Spreer, 1998). Utilization of whey proteins for industrial uses is important not only for the complete utilization of raw milk in the cheese making process, but also to reduce the biological oxygen demand (BOD) load of wastewater (Spreer, 1998).

Liquid whey consists of only 0.6% protein with a majority of its weight (93%) being water. Dried whey contains approximately 13% protein, 76% lactose, 10% ash, and 1% fat (Huffman, 1996). Whey proteins (Table 1) are mainly composed of two gene products including β-lactoglobulin and α-lactalbumin (Marshall, 1982).

Table 1 – Protein components of liquid whey proteins[a].

Protein	Content (mg/mL)
β-lactoglobulin	3.0
α-lactalbumin	0.9
Serum albumin	0.3
Lactoferrin	0.012
Lysozyme	0.0001
Immunoglobulins (Ig)	
IgG	0.6
IgA	0.03
IgM	0.03

[a] Marshall (1982)

β-Lactoglobulin is a heat labile protein that accounts for the majority of the proteins in the whey. α-Lactalbumin is biologically involved in the regulation of lactose biosynthesis and makes up the second largest portion of the whey component. Lactoferrin can bind iron, similar to transferrin in the blood, and is used as a transport mechanism for iron (Marshall, 1982). Lysozyme has the ability to degrade bacterial cell

walls, giving it anti-microbial activity (Marshall, 1982). The biological activities of these proteins may have some effect when added as an ingredient in a meat system.

The overall composition of whey varies based on the method used to separate it from the casein portion of the milk (Marshall, 1982). Table 2 highlights these differences:

Table 2. Composition and pH of various caseins and whey proteins[a].

	Rennet whey (g/L)	Lactic whey (g/L)	Acid whey (g/L)
Total solids	66	64	63
Protein (N x 6.8)	6.2	5.8	5.8
Non-protein nitrogen	0.37	0.40	0.30
Lactose	52.3	44.3	46.9
Minerals (as ash)	5.0	7.5	7.9
Milk fat	0.2	0.3	0.3
Phosphate	1.0	2.0	2.0
Calcium	0.5	1.6	1.4
Sulfate	0.7	0.5	2.8
Magnesium	0.07	0.10	0.11
Sodium	0.53	0.51	0.50
Potassium	1.45	1.40	1.40
Chloride	1.02	0.9	0.9
Lactate		6.4	
pH	6.4	4.6	4.7

[a] Marshall (1982)

Variations in these components may have an affect on meat products when these proteins are used as an ingredient, however, no research on this possible interaction was found.

2.1.3 Dairy proteins in meat products

There are many commercial applications for dairy proteins in meat products. However, many of the specific uses are proprietary and are not discussed in literature (Mulvihill, 1991). Dairy proteins can be added to meat products as binders and are regulated according to Title nine of the Code of Federal Regulations (USDA, 1998).

However, dairy proteins can have significant functional properties when added to meat products that can make their addition even more beneficial. Table 3 summarizes the permitted levels of dairy proteins in meat and poultry products as detailed in the Code of Federal Regulations.

Table 3 – Approved usage and usage levels for various dairy proteins[a].

Ingredient	Purpose	Products	Level
Calcium reduced dried skim milk	Bind and extend	Sausages	3.5% individually, or collectively with other binders
Dried milk	Bind and extend	Sausages	3.5% individually, or collectively with other binders
Sodium caseinate	Bind and extend	Imitation sausages	Sufficient for purpose
		Sausages	2.0%
Dried whey, reduced mineral whey, or WPC	Bind or thicken	Sausages, restructured meat products, whole muscle meat cuts	3.5% individually, or collectively with other binders
		Imitation sausages, non-specific loaves	Sufficient for purpose

[a] From the Code of Federal Regulations 9CFR318.7 (USDA, 1998)

Functional properties. In general, dairy proteins can be incorporated into meat products to improve the water holding capacity, improve texture, and improve color. These improvements in product quality are related to specific functional properties that vary among the numerous types of dairy proteins available. The following is a general overview of functional properties of dairy proteins in meat products. Functional properties of specific proteins also will be discussed in later sections.

Dairy proteins, when added to meat systems, can improve the water holding capacity. In typical processed meat products, salt and phosphates are added in a brine solution to extract the salt-soluble proteins (SSP), especially myosin (Schmidt, 1987).

The extracted SSP act to bind the meat pieces upon cooking. During the cooking process, the extracted proteins also bind water. Dairy proteins when added to the meat system can increase the amount of water binding sites and therefore increase the capacity for water retention (Huffman, 1996).

Similar to increasing water retention of cooked meat products, the extracted meat proteins are important for fat emulsification. During cooking, lipid droplets must be stabilized by the extracted proteins to avoid fat separation. Separation of the fat component from the meat matrix can cause fat pockets within or on the surface of the meat product (Schmidt, 1987). Dairy proteins can be used to stabilize the meat emulsion and prevent fat migration. Additionally, if dairy proteins are used to stabilize the fat, the extracted meat proteins are more available for water binding (Hoogenkamp, 1986)

Milk proteins can also be added to meat systems to enhance gel formation upon heating (Hoogenkamp, 1986). Gel formation in meat products is an important factor in the texture of the meat products.

Another property that dairy proteins can provide in some food products is their high nutritional value (Hambraeus, 1982). However, since meat products are already an excellent source of dietary proteins and the essential amino acids, this property is not of significant importance.

Methods of incorporating dairy proteins. The addition of dairy proteins to meat products faces some challenges that must be addressed. Because of some solubility and caking problems, several methods of adding dairy proteins to meat products have been developed. Dairy proteins can be added directly to the meat as a powder or by the addition of a jelly where 10-15% milk protein has been combined with water.

Alternatively, a pre-emulsified protein-fat mixture can also be used to incorporate the dairy proteins (van den Hoven, 1987). Brine solutions containing 0.5 to 1% dairy proteins can also be added to marinated or pumped meat products (Hoogenkamp, 1989). Care must be taken to avoid clumping of the proteins which could cause clogging of the injection needles (Hoogenkamp, 1989). Commercial mixers are available that can adequately solubilize the protein in the brine prior to addition to the meat product.

A two-step method for combining the functional ingredients such as dairy proteins, carrageenan, soy proteins, and food starches in a brine solution was reported by Hoogenkamp (1989). In this method, the dairy proteins are added to a portion of the water in addition to the salt. The phosphates are dissolved separately in water with other ingredients (soy, carrageenan, etc.) following. The dairy protein solution is then slowly combined with the phosphate solution.

The addition of dairy proteins during the tumbling process can be used for products where some size reduction of the meat pieces has already been done. In this type product the dairy proteins become fully solubilized and are drawn into the meat. This process can be aided by maceration of the large meat pieces prior to tumbling (Hoogenkamp, 1989).

2.1.4 Specific dairy proteins

Nonfat dry milk (NFDM). Nonfat dry milk, or skim milk powder is produced from fresh, high quality milk using a basic set of procedures. The fat of whole milk is removed and redirected to other uses. The skim milk is pasteurized to destroy all pathogenic organisms, evaporated to increase solids, and spray or roller dried to a dry powder, and packaged (Caríc, 1994). Three heat classifications of nonfat dry milk

include low heat, medium heat and high heat. These classifications are based on the amount of denatured whey protein in the milk powder. High heat nonfat dry milk will have a greater amount of denatured whey protein than the low heat product (Caríc, 1994). Milk powder can be "instantized" to improve reconstitution properties of the powder by an agglomeration process that results in a powder that will dissolve quickly upon addition to water.

Nonfat dry milk powder contains similar proteins and minerals found in raw milk (Table 4). Concentration of the various components are higher in the dried product because the water is removed.

Table 4 – Components of whole milk and nonfat dry milk

	Whole milk (g/100g)	Nonfat dry milk (g/100g)
Water	87.5	4.3
Protein (N x 6.8)	3.13	35.0
Carbohydrates	4.84	51.90
Ash	0.8	7.80
Milk fat	3.76	0.97
Phosphorus	0.09	1.02
Calcium	0.13	1.29
Sodium	0.05	0.56
Potassium	0.16	1.58
Chloride	0.09	-

[a] Caríc (1994)

Nonfat dry milk is added to certain meat products as a binder or an extender. Dobson et al. (1993) reported that nonfat dry milk increased the bind strength of turkey rolls when added at 3% in both coarse and fine emulsions.

Casein and caseinates. The manufacturing process of caseins involves coagulation of the proteins by the reduction of pH or by enzymatic action. Lactic acid producing bacteria can be added to the milk to convert the naturally occurring lactose into lactic acid, thus reducing the pH. Casein made by this procedure would be known as

lactic casein. Alternatively, the pH can be reduced by the addition of acids, such as hydrochloric, sulfuric, or lactic acid. Proteins derived from this process would be named for the acid used in the coagulation. For example, protein made through the addition of hydrochloric acid would be known as hydrochloric acid casein (Muller, 1982). The enzymes chymosin (rennin) and pepsin can also be used to coagulate the proteins by disrupting the casein micelles in the presence of calcium ions (Muller, 1982).

Caseinates are produced by the addition of strong alkaline solutions such as sodium hydroxide and calcium hydroxide to produce sodium caseinate and calcium caseinate, respectively. In both cases, a fine slurry of casein proteins is mixed with the appropriate alkali and dried by spray or roller dryers. While sodium and calcium caseinates are the most common types used in food applications, others such as citrated, ammonium, potassium and magnesium caseinates also can be produced (Muller, 1982).

Functional properties. Although allowed in meat products as a binder or an extender, caseinates can improve fat emulsification in meat products because they contain both high electrical charges and several hydrophobic groups (van den Hoven, 1987). During particle size reduction, shear forces can disturb the fat droplet causing the fat to migrate through the meat system to develop fat pockets within the product (Andres, 1985). In addition, if milk proteins are used to stabilize the fat in the meat system, the salt solublized myofibrillar proteins are free to bind water and provide structure within the meat product. This could result in better yield of products and therefore could have a positive economical impact (Andres, 1985).

Sodium caseinate, while not gel forming, can improve the gel strength of meat products, whereas calcium caseinates may actually disrupt the gel formation in meat

products (van den Hoven, 1987). Calcium caseinates also can be used for a whitening effect in products made with high percentages of turkey thigh meat (van den Hoven, 1987).

An interesting use for dairy proteins in finely comminuted meat products is the production of pre-made protein/fat emulsions (Andres, 1985; van den Hoven, 1987). In this process, lower quality fats such as chicken fat, beef suet and tallow, pork leaf fat, and vegetable oils can be added to meat products as a pre-emulsified blend. During cooking the fat remains in the product due to the caseinate's resistance to denaturation and the ability to stabilize the fat particles.

The ability of sodium caseinate to increase cooking yield and decrease product shrinkage has been reported in several works. Jantawat and Carpenter (1989) reported that sodium caseinate increased cooking yield in a smoked poultry sausage product without affecting the firmness, chewiness, or springiness of the product. The authors speculated that increased water and fat binding were responsible for the increase in yield. Both Camou and Sebranek (1991) and Mills (1995) found similar increases in yield for pork products. In addition, Mills (1995) reported that sodium caseinate decreased purge in vacuum packaged ham slices. This could be an important factor in pre-sliced deli meats, that are now common in grocery stores. Mills (1995) also reported a decrease in shear force for products formulated with sodium caseinate, potentially having a detrimental effect on product acceptability. However, in this study, a sensory panel did not find any difference in texture.

Whey and WPC. Whey is a liquid by-product of cheese or casein production containing only 0.6% protein with a majority of its weight (93%) being water (Huffman,

1996). Dried whey contains approximately 13% protein, 76% lactose, 10% ash, and 1% fat. To produce whey protein concentrates (WPC), the liquid whey can be concentrated before drying using ultrafiltration or diafiltration with typical protein levels from 35% to 80%. Other processes such as enzymatic hydrolysis of lactose and microfiltration can be used to reduce lactose and lipid levels before drying. Lactalbumin is a specific type of WPC where the proteins have been heat denatured and concentrated to 80% to 90% protein (Huffman, 1996). Whey protein isolates, with typically greater than 90% protein, undergo both lipid and lactose reduction before drying (Huffman, 1996). Demineralization by electrodialysis or ion exchange can be used to reduce the mineral content of the whey before drying (Caríc, 1994). Electrodialysis removes the minerals by use of an ion-selective semipermiable membrane. Ion-exchange utilizes both a cation exchanger, where positively charged ions are replaced with hydrogen ions (H^+), and an anion exchanger where negatively charged ions are replaced with hydroxide ions (OH^-) (Caríc, 1994).

Functional properties. Whey can be produced to have a variety of functional properties. In cooked meat products, WPC can affect gelation within the meat system. The formation of a gel in meat products during cooking can allow for greater water holding capacity, stabilize the cooked meat, improve texture and color (van den Hoven, 1987). Gelation properties are dependent on the pH, protein concentration, and other ingredients (Huffman, 1996). The gelation occurs during cooking as the proteins unfold and build intermolecular disulfide bonds (van den Hoven, 1987). There are some conflicting thoughts on what effect WPC has on the gelation within a meat system. Some

research finds that WPC interferes with the normal gelation in the meat system (Hoogenkamp, 1986).

Dobson et al. (1993) reported that WPC at 3% increased the bind strength of a finely chopped emulsion type product. El-Magoli et al. (1996) reported an increases in cooking yield and fat retention along with a decrease in shrinkage as WPC concentration was increased. El-Magoli et al. (1995) and Hung and Zayas (1992) also found increases in cooking yield due to WPC incorporation in meat products.

2.2 PINK COLOR DEFECT

2.2.1 Nitrite or nitrate contamination

Many causes have been associated with this pink color development. Nitrite in fresh meat products, regardless of the source, has the potential to cause a pink color in turkey products due to the formation of nitrosylhemochrome (NITHEME), a heat stable pink pigment. NITHEME is the typical pigment associated with cured meats where nitrite is intentionally added. Curing, mainly a preservation method, also provides meat products with a “cured” flavor and a heat stable pink color. While the concentration of nitrite in cured products is relatively high, Ahn and Maurer (1987) reported that as little as 1 ppm nitrite in turkey caused a pink color. With such a low amount causing a pink color, extreme care must be taken to avoid any nitrite contamination. However, many sources of nitrite have been identified.

Ahn and Maurer (1987) reported that the level of nitrate and nitrite in the raw turkey breast ranged from 0 to 0.7 ppm nitrite and 3.8 to 21 ppm nitrate. Mesophilic

organisms can convert nitrate into nitrite (Ahn and Maurer, 1987) potentially causing a pink color.

Froning et al. (1969b) reported higher visual redness scores with increased levels of nitrates and nitrites in feed. The visual difference was not apparent in hot samples, but was evident after cooling. Nitrites or nitrates in cooling water also can cause a pink color development in meat if adequate contact is allowed. Nash et al. (1985) reported that storing chicken carcasses in water containing 3 ppm nitrite or 50 ppm nitrate at 4°C increased redness. Although 3 ppm was the lowest level of nitrite tested, the data suggested that lower levels may have also given a pink color.

Other pigments have also been associated with the pink color development. Girard et al. (1990) found that cytochrome c was responsible for the pink color in both turkey breasts and pork loins. Cytochrome c, a heat stable pink pigment, was identified by both spectral analysis and SDS-Page analysis. Ahn and Maurer (1990a) reported that reduced cytochrome c was bright pink in color.

Brown and Tapple (1957) determined that denatured hemochrome complexes were responsible for the pink color of cooked and canned tuna. Because nicotinamide is able to react with the heme containing pigments and is found at relatively high concentrations in tuna, nicotinamide hemochrome (NICHEME) was suspected as the pink pigment.

Howe et al. (1982) reported a pink color that formed in cooked pork loin roasts faded quickly upon exposure to light and oxygen loins. Ghorapade and Cornforth (1993) found that denatured globin hemochrome complexes were the cause of the pink color

development in cooked pork roasts. The authors also reported that the pink color faded quickly upon exposure to air.

Cornforth et al. (1986) attributed the sporadic pink color development in turkey to hemochrome complexes. And because nicotinamide is also found in turkey at relatively high concentrations, it has been speculated that NICHEME is responsible for this heat stable pink color. In addition, the authors found that reflectance patterns from commercially produced pink samples were similar to the reflectance patterns of experimental samples containing nicotinamide. Reducing conditions within the meat block were reported to be important in the formation of the hemochrome-complexes.

Upon testing potential ligands that bind to heme containing pigments to form heat stable pink pigments, Ahn and Maurer (1990a) reported that of the potential ligands tested, nicotinamide was one of best at forming a heme-complex. Claus et al. (1994) reported that 2.0% nicotinamide increased CIE a* values, lowered b* values but did not affect L* values.

2.2.2 Other causes for pink color in cooked meats

Many other factors also have been reported to cause a pink color in meat products. Froning (1969a) reported that roasted meat from chickens and turkeys exposed to automobile exhaust prior to slaughter was more red than control birds. This study used very high concentrations of exhaust fumes, and may not have been indicative of normal levels that birds are exposed to during transport. However, the amount of time that birds are exposed to these fumes may also be a factor in developing a red color. During long delays in transportation or off-loading, birds located close to the transport truck's exhaust may be exposed to these fumes for extended periods of time.

End point cooking temperature also can be an important factor in the color of cooked meat (Claus et al., 1994). Helmkey and Froning (1971) reported that redness of cooked turkey products decreased and lightness increased as end-point cooking temperature increased from 60 to 82°C. Using spectral data, the authors concluded that the pink pigment in samples cooked to 60 to 77°C was oxymyoglobin. Ang and Huang (1994) reported that as end point temperature increased in cooked chicken leg patties, lightness increased and both yellowness and redness decreased. Claus et al. (1994) reported that redness of turkey increased with decreased chilling rates.

The oven gases, carbon monoxide or nitric oxide from gas fired ovens also have been blamed for causing a pink color development (Pool, 1956). Ammonia exposure to raw meat has been shown to increase redness in cooked meat (Shaw et al., 1992).

Young et al. (1996) determined that aging of turkey breast muscles for less than 8 hours resulted in less myoglobin denaturation and a higher degree of red color. Yang and Chen (1993) found that redness of cooked chicken meat increased over a 28-day storage time of the raw meat. The pH of the raw meat also increased over the storage time, possibly affecting the denaturation of the myoglobin during cooking.

2.3 COOKED MEAT PIGMENTS

Denatured globin hemichrome, the brown pigment associated with typical cooked meat, is the result of denaturation of the heme containing proteins during cooking (Fox, 1987). The iron within the hemichromes is in the ferric (Fe^{+++}) oxidation state. Tapple (1957) proposed that a mixture of nicotinamide hemichrome along with the denatured globin hemichrome combine to give the overall brown color of cooked meats. It was

determined that under reducing conditions, hemochromes, specifically denatured globin and nicotinamide hemochromes formed, resulting in a pink color. In this form, the oxidation state of the heme iron is in the ferrous (Fe^{++}) form.

2.3.1 Effects of additives and storage on cooked meat color

Phosphates. Phosphates are added to meat products to aid in the extraction of salt soluble proteins which are important to increasing bind. Ahn and Maurer (1989a) found that phosphates increased the amount of residual nitrites and decreased lightness in samples that were prepared with varying levels of sodium nitrite. Phosphates increased redness values (Ahn and Maurer, 1989b). Phosphates increased the heat stability of myoglobin and decreased heat stability of cytochrome c (Ahn and Maurer, 1989c). High pH (>6.4) values were reported to be most favorable for heme-complex forming reactions where amino acids or proteins and pigments are the main reactants (Ahn and Maurer, 1990b)

Salt (sodium chloride). Ahn and Maurer (1989a and 1989b) reported that salt increased redness values (a) while it decreased the lightness (L) and the yellowness. Salt was also reported to decrease the heat stability of myoglobin but increase the heat stability of cytochrome c (Ahn and Maurer, 1989c).

Other potential additives. Dextrose was reported to increase the heat stability of myoglobin heated at 68°C and cytochrome c heated at 85°C. Froning et al. (1968) reported that egg white solids increased redness of cooked turkey meat samples when added at a 10% level. The egg whites increased the pH of the turkey meat, potentially influencing the color differences.

Effect of storage time. Froning et al. (1969b) found that, upon refrigeration, a pink color developed in samples where dietary nitrates and nitrites were fed to birds. The authors speculated that the pink color regeneration was due to reversal of the denaturation process.

Storage time of 5 to 10 days was found to increase visual red color in turkey samples that were cooked to various end-point temperatures (Helmke and Froning, 1971). However, the instrumental measurements of red color did not agree with the "visual" assessment. When evaluating a pink color development in pork, Howe et al. (1982) did not find storage time to be a significant factor in increasing pink color. It was speculated that the reaction to form pink pigments occurs rapidly upon cooling, but did not increase over time. Claus et al. (1994) reported that samples containing 2.0% nicotinamide increased in redness over storage time.

The following three research chapters have been prepared for journal publication. Although the chapters are interrelated, each was prepared from different research projects.

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Chapter 3

Determination of Nicotinamide Hemochrome and Nitrosylhemochrome by Reflectance Spectrophotometry in Cooked Ground Turkey

3.1 ABSTRACT

Nitrosylhemochrome (NITHEME) and nicotinamide hemochrome (NICHEME) have been associated with pink color development in cooked turkey breast. The objective of this experiment was to determine if spectrophotometric reflectance ratios were capable of predicting changes in these pigments. The cubic model fit of two reflectance ratios was determined for added nicotinamide (0 to 2.0%) and sodium nitrite (0 to 20 ppm). The ratio %R 537 nm divided by %R 553 nm was able to predict NICHEME (R^2=0.99) and the ratio %R 650 nm divided by %R 570 nm predicted NITHEME (R^2=0.97). NICHEME content may cause interference in NITHEME measurement using this method as noted by a relatively high fit (R^2=0.87) for the NITHEME predictor in samples containing nicotinamide.

3.2 INTRODUCTION

Many causes have been associated with a sporadic pink color development in cooked uncured turkey and other meat products. Nitrite or nitrate contamination, whether related to live animal consumption in feed (Froning et al., 1969) or later during processing (Ahn and Maurer, 1987; Mugler et al., 1970, Nash et al., 1985) was thought to be the cause of the pink color. Other factors have also been implicated including carbon monoxide or nitric oxide in oven gasses (Poole, 1956), stability of myoglobin (Trout, 1989; Young et al., 1996) or cytochrome c (Girard et al., 1990) to heat denaturation, and

development of various hemochromes (Ghorpade and Cornforth, 1993; Cornforfth et al., 1986). All of these causes have been shown to increase the red color in experimental samples.

Nitrite has long been recognized to responsible for a pink color development in meat products due to its role in the curing process. Nitrite added to meat products, whether intentional or accidental, forms nitric oxide, which can react with the heme-containing pigments and upon cooking forms nitrosylhemochrome (NITHEME), a heat stable pink pigment (Hotchkiss, 1987). However, most of the recent research, based on pigment extraction and spectrophotometric techniques, point to non-nitrosyl pigments as the cause of the sporadic pink color development in turkey and pork products (Cornforth et al. 1986).

Hemochromes are cooked meat pigments that can form when the heme iron is in the ferrous (Fe++), or reduced, form (Fox, 1987). Both NITHEME and denatured globin hemochrome are bright pink in color. Brown and Tapple (1957) speculated that the pink pigment of canned tuna was a mixture of nicotinamide hemochrome (NICHEME) and denatured globin hemochrome. Both denatured globin and nicotinamide have the ability to react and bind with the heme-containing pigments during cooking to form heat stable pink pigments in a manner similar to the NITHEME formation. Cornforth et al. (1986) determined that the pink color development in turkey rolls was likely due to NICHEME development because the spectral patterns of commercially pink products were very similar to those of laboratory samples that were made with added nicotinamide.

Reflectance spectrophotometry has been used extensively to predict specific pigment types in both raw and cooked meat products (AMSA, 1991). Schwarz et al.

(1998) reported that reflectance (R) wavelength ratio of %R at 537 nm divided by %R at 553 nm had a high correlation to NICHEME and did not respond to variations in NITHEME content. However, the low correlation of the %R at 537 nm divided by %R at 553 nm ratio to added nitrite may have been due to the use of only three levels of added sodium nitrite (0, 75, and 150 ppm) and the relatively high concentrations of added nitrite. It is possible that at high levels of added nitrite all of the available pigments were bound, creating no additional NITHEME and a leveled-off curve. In this situation, the linear relationship of any measurement technique would be a flat line and result in a low correlation.

NITHEME, the heat stable pigment in cured meat products, has been evaluated using the reflectance ratio of %R at 650 nm divided by %R at 570 nm (Kraft and Ayres, 1954; Erdman and Watts, 1957; AMSA, 1991). This ratio was established to compare levels of cure in meat products where the level of nitrite is relatively high. Consequently, its ability to predict low levels of NITHEME, those that may be associated with nitrite contamination, has not been evaluated.

The objective of this experiment was to provide support of the recent work done by Schwarz et al. (1998) on reflectance spectrophotometric methods for determining NICHEME by the addition of nicotinamide at levels up to 2.0%. In addition, the NITHEME predictor, %R at 650 nm divided by %R at 570 nm, was also examined in samples containing up to 100 ppm sodium nitrite. To more closely examine the ability of the reflectance ratios to measure pigments at contamination levels, samples were prepared with lower concentrations of the pink color generating ligands, nicotinamide and sodium nitrite.

3.3 MATERIALS & METHODS

3.3.1 Sample preparation

Boneless turkey breast muscles (*Pectoralis major*) obtained from a Virginia poultry processor were ground once through a 1.27-cm plate (model 4532, The Hobart Mfg. Co., Troy, OH), and mixed for 4 min (model A-200, The Hobart Mfg. Co., Troy, OH) with dough hook accessory. Meat for each of three replications, obtained on different production days was split into portions designated for the ligand treatments (sodium nitrite and nicotinamide). Individual portions of the coarse-ground meat were vacuum packaged and frozen in moisture impermeable barrier bags (item 030056, Koch Supplies and Equipment Co., Kansas City, MO). For each ligand treatment, one portion of the frozen turkey was allowed to thaw at 2 to 4°C for 48 to 60 h. The tempered meat was then ground twice through a 4.76-mm plate.

Solutions containing 2.0% sodium chloride, 0.5% sodium tripolyphosphate, and the ligands (various levels of sodium nitrite or nicotinamide) were added to the ground turkey breast muscle at a level of 30% (all amounts were calculated on a meat weight basis). The following levels of ligands were randomly assigned to individual meat mixtures; nitrite at 0, 1.5, 3, 4.5, 6, 7.5, 10, 15, 20, 50, 75, and 100 ppm and nicotinamide at 0.0, 0.1, 0.18, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 2.0 percent. Upon addition of the salt/phosphate and ligand solutions, samples were mixed for approximately one min using a hand mixer (model KHM3WH-1, Kitchen Aid, St. Joseph, Michigan). The turkey mixtures were stuffed into 50 mL plastic test tubes, capped, and centrifuged (model PR-2, International Equipment Company) for ten min at 2000 x G to remove air pockets from the product.

The samples were stored at 2 to 4°C overnight in the dark to allow adequate time for ingredient interactions and then cooked in an 85°C circulating water bath (custom built, Virginia Tech, Blacksburg, VA) to an internal temperature of 80°C. The samples were immediately cooled for 30 min in an ice bath and stored overnight in a 2 to 4°C cooler. Internal temperature of the products was monitored throughout the cooking process by placing four randomly located thermocouple-containing samples attached to a datalogger (model 5100, Electronic Controls Design, Milwaukee, Oregon) throughout the water bath.

3.3.2 Sample treatment and instrumental measurement

Instrumental measurements were taken on freshly exposed samples within approximately 10 sec of cutting. CIE L*, a*, and b* values were measured using a chroma meter (model CR-200, Minolta Corp., Osaka, Japan). A UV/Visible scanning spectrophotometer (model 2101PC, Shimadzu Inc., Kyoto, Japan) was used to obtain reflectance spectra from 400 nm to 700 nm. The instrument was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm and fast scan speed. Both instruments were calibrated using a white calibration plate (L* 97.91, a* -0.68, b* 2.45).

The reflectance ratio of % R at 537 nm divided by % R at 553 nm was used to predict NICHEME in the no ligand and nicotinamide treated samples (Schwarz et al., 1998). NITHEME was determined in the nitrite containing samples by the reflectance ratio of %R at 650 nm divided by %R at 570 nm (Erdman and Watts, 1957; Kraft and Ayres, 1954; AMSA, 1991). For both ratios, higher values were associated with higher pigment levels.

Pigment manipulations as reported in Erdman and Watts (1957) and Cornforth et al. (1986) were used to chemically alter muscle pigments on one sample from each treatment. Samples were exposed to solutions of potassium ferricyanide (PF, 0.1%), an oxidizing agent; sodium dithionite (SD, 0.1M), a reducing agent; and hydrogen peroxide (HP, 0.05%), which breaks the porphyrin ring and renders the pigments colorless (Erdman and Watts, 1957). Samples were incubated at room temperature in the appropriate solutions for 4 h PF, 20 min HP, and 10 min SD. Air and light exposed samples were placed under 1076 lux fluorescent lighting (model F20T12/30U, Phillips Lighting Co., Somerset, NJ) for 2 min. Color and reflectance spectrophotometric measurements were repeated on all of the chemically altered samples.

3.3.3 Statistical analysis

Since it is hypothesized that there is a non-linear relationship between color and level of ligand added, the cubic model $Y=\beta_0 + \beta_1(\text{level}) + \beta_2(\text{level})^2 + \beta_3(\text{level})^3$ was used to determine the multiple regression fit using SAS (1990) on responses from all levels of added nicotinamide and on responses from 0 to 20 ppm added sodium nitrite. Interest focused on estimating and testing the adequacy of the fit for a variety of conditions and treatment combinations.

3.4 RESULTS & DISCUSSION

3.4.1 Fresh-cut samples

To simulate levels that could be associated with nitrite or nitrate contamination, it was determined that responses from samples containing greater than 20 ppm added

sodium nitrite would not be used in the multiple regression analysis. This was based on the results of the NITHEME predictor (%R650nm/%R570nm) and CIE a* values increased up to approximately 10 to 20 ppm of added sodium nitrite and then leveled off at higher concentrations. In contrast, CIE a* values and the NICHEME predictor (%R537nm/%R553nm) increased over the entire tested range of added nicotinamide so all levels were used in the analysis.

The reflectance ratio of %R537nm/%R553nm was a good predictor for NICHEME (Fig. 1, R^2=0.99) in the fresh-cut samples prepared with various nicotinamide treatments, supporting the work of Schwarz et al. (1998). When using this ratio to evaluate samples produced to contain sodium nitrite, the cubic fit was unexpectedly high (Fig. 2, R^2=0.68) in contrast to a very low correlation (r=0.029) reported by Schwarz et al. (1998). However, from our results it became apparent that the fit was almost a flat line with very little variation (Fig. 2). The NICHEME predictor (%R537nm/%R553nm) would thus show very little interference from slight changes in NITHEME content in fresh-cut samples.

As expected, the ratio of %R650nm/%R570nm was a good estimator of NITHEME (Fig. 3, R^2=0.97), but may also be affected by NICHEME content (Fig. 4, R^2=0.87). This offers the possibility that naturally occurring variations of NICHEME among turkey samples may interfere with the NITHEME predictor (%R650nm/%R570nm).

CIE a* values were used to compare pink color development in the samples related to increased nicotinamide or nitrite in the samples. In samples containing nicotinamide (Fig. 5) or sodium nitrite (Fig. 6), CIE a* values increased with increasing

concentration of added ligand. Although not illustrated, CIE a* values leveled off at concentrations over 20 ppm of added sodium nitrite. This was possibly due to a saturation of available pigments with which the nitric oxide can bind. CIE a* values had a high fit to the cubic model in both added nicotinamide (R^2=0.97) and sodium nitrite (R^2=0.98). These results confirmed that the ligand treatments were effective at inducing red coloration in the turkey samples.

3.4.2 Treated samples

The results of the treated samples along with model information are summarized in Table 1. The NICHEME predictor (%R537nm/%R553nm) was also a good predictor of reduced NICHEME (R^2=0.94) and light and air exposed NICHEME (R^2=0.99), while not predicting oxidized (R^2=0.43) and H_2O_2 (R^2=0.41) treated NICHEME. The low prediction of the oxidized and the H_2O_2 treated NICHEME samples was expected because the treatments were designed to either convert the NICHEME to its oxidized form, nicotinamide hemichrome, which is brown in color, or break the heme ring. Unfortunately, the NICHEME predictor also had a high fit (R^2=0.95) to the light and air exposed nitrite containing samples. But again the response was virtually a flat line, and would therefore not lead to interference of the NICHEME measurement.

The NITHEME predictor (%R650nm/%R570nm) did predict all forms of NITHEME including light and air exposed (R^2=0.97), oxidized (R^2=0.94), reduced (R^2=0.98) with only marginal prediction of NITHEME in H_2O_2 treated samples (R^2=0.66) (Table 1). The NITHEME predictor also predicted light and air exposed (R^2=0.95) and reduced (R^2=0.93) pigments in the samples with nicotinamide. This further supports the

potential for the NITHEME predictor to have an interference with the various forms of NICHEME tested.

3.5 CONCLUSION

The reflectance ratio of %R537nm/%R553nm was a good predictor of NICHEME. This NICHEME predictor did not substantially change over the range of NITHEME tested indicating that incidental presence of nitrite will not interfere with the prediction of NICHEME.

The reflectance ratio of %R650nm/%R570 was able to predict NITHEME in turkey even at low levels of added sodium nitrite. However, this ratio did display some response from added nicotinamide. This offers the possibility that natural variations in nicotinamide levels among turkey samples could interfere with the measurement of NITHEME using the reflectance spectrophotometric methodology. Consequently, when using this measurement technique for NITHEME, researchers should be cautious to control for NICHEME development in experimental products.

This research demonstrated the ability for the reflectance ratios to correctly predict the various levels of added pink generating ligands when it was known which ligand was added. However, when the sporadic pink color development occurs in the poultry industry, the cause is not usually known. Additional research is needed to determine if other reflectance ratios can be used to differentiate between NICHEME and NITHEME without interference.

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Table 1 - Determination of nicotinamide hemochrome (NICHEME) and nitrosylhemochrome (NITHEME) using reflectance spectrophotometric ratios.

Ligand[a]	Predictor[b]	Treatment	Cubic Model[c]	R^2
NIC	**NICHEME (537/553)**	Fresh-cut	$y= 1.007^{***} + 0.011(LEV) + 0.028(LEV)^2 - 0.008(LEV)^3$	0.99
		Reduced	$y= 1.128^{***} + 0.467^{**}(LEV) - 0.370^{*}(LEV)^2 + 0.093^{*}(LEV)^3$	0.94
		Light-air exposed	$y= 1.005^{***} + 0.016(LEV) + 0.014(LEV)^2 - 0.004(LEV)^3$	0.99
		Oxidized	$y= 0.988^{***} + 0.015(LEV) - 0.016(LEV)^2 + 0.005(LEV)^3$	0.43
		H_2O_2 treated	$y= 0.999^{***} - 0.002(LEV) + 0.002(LEV)^2 - 0.000(LEV)^3$	0.41
	NITHEME (650/570)	Fresh-cut	$y= 1.176^{***} + 0.123^{*}(LEV) - 0.091(LEV)^2 + 0.023(LEV)^3$	0.87
		Reduced	$y= 1.293^{***} - 0.204^{**}(LEV) + 0.187^{**}(LEV)^2 - 0.052^{*}(LEV)^3$	0.93
		Light-air exposed	$y= 1.178^{***} + 0.041(LEV) + 0.010(LEV)^2 - 0.007(LEV)^3$	0.95
		Oxidized	$y= 1.140^{***} + 0.018(LEV) - 0.018(LEV)^2 + 0.006(LEV)^3$	0.32
		H_2O_2 treated	$y= 1.065^{***} - 0.005(LEV) + 0.004(LEV)^2 - 0.003(LEV)^3$	0.66
NIT	**NITHEME (650/570)**	Fresh-cut	$y= 1.167^{***} + 0.025^{***}(LEV) - 0.002^{*}(LEV)^2 + 3.5 \times 10^{-5} (LEV)^3$	0.97
		Reduced	$y= 1.258^{***} + 0.026^{***}(LEV) - 0.002^{**}(LEV)^2 + 3.8 \times 10^{-5}{}^{*}(LEV)^3$	0.98
		Light-air exposed	$y= 1.169^{***} + 0.024^{***}(LEV) - 0.001(LEV)^2 + 2.4 \times 10^{-5} (LEV)^3$	0.97
		Oxidized	$y= 1.142^{***} + 0.010^{**}(LEV) - 0.001(LEV)^2 + 1.4 \times 10^{-5} (LEV)^3$	0.94
		H_2O_2 treated	$y= 1.058^{***} + 0.021^{**}(LEV) - 0.001^{*}(LEV)^2 + 3.3 \times 10^{-5} (LEV)^3$	0.66
	NICHEME (537/553)	Fresh-cut	$y= 1.007^{***} + 0.001(LEV) - 2.3 \times 10^{-5} (LEV)^2 - 1.1 \times 10^{-6} (LEV)^3$	0.68
		Reduced	$y= 1.042^{***} - 0.004(LEV) + 4.4 \times 10^{-4} (LEV)^2 - 1.4 \times 10^{-5} (LEV)^3$	0.65
		Light-air exposed	$y= 1.004^{***} + 0.002^{*}(LEV) - 3.3 \times 10^{-5} (LEV)^2 - 2.0 \times 10^{-6} (LEV)^3$	0.95
		Oxidized	$y= 0.994^{***} - 4.1 \times 10^{-4} (LEV) + 2.7 \times 10^{-5} (LEV)^2 - 6.7 \times 10^{-7} (LEV)^3$	0.27
		H_2O_2 treated	$y= 0.998^{***} + 0.002^{*}(LEV) - 2.3 \times 10^{-4} (LEV)^2 + 6.8 \times 10^{-6} (LEV)^3$	0.41

[a] Nicotinamide (NIC) was added at 10 levels from 0 to 2.0%; sodium nitrite (NIT) was added at 9 levels from 0 to 20 ppm (based on meat weight)

[b] The reflectance ratio of %R 537 nm/%R 553 nm was used to predict NICHEME; the ratio of %R 650 nm/%R 570 nm was used to predict NITHEME. The pigment predictors were utilized on samples containing both added nicotinamide and sodium nitrite, where higher values are associated with higher pigment levels.

[c] The cubic model $Y=\beta_0 + \beta_1(level) + \beta_2(level)^2 + \beta_3(level)^3$ was used to determine the multiple regression fit (R^2). Significance of the model coefficients are denoted as *** (P<0.001), ** (P<0.01), and * (P<0.05).

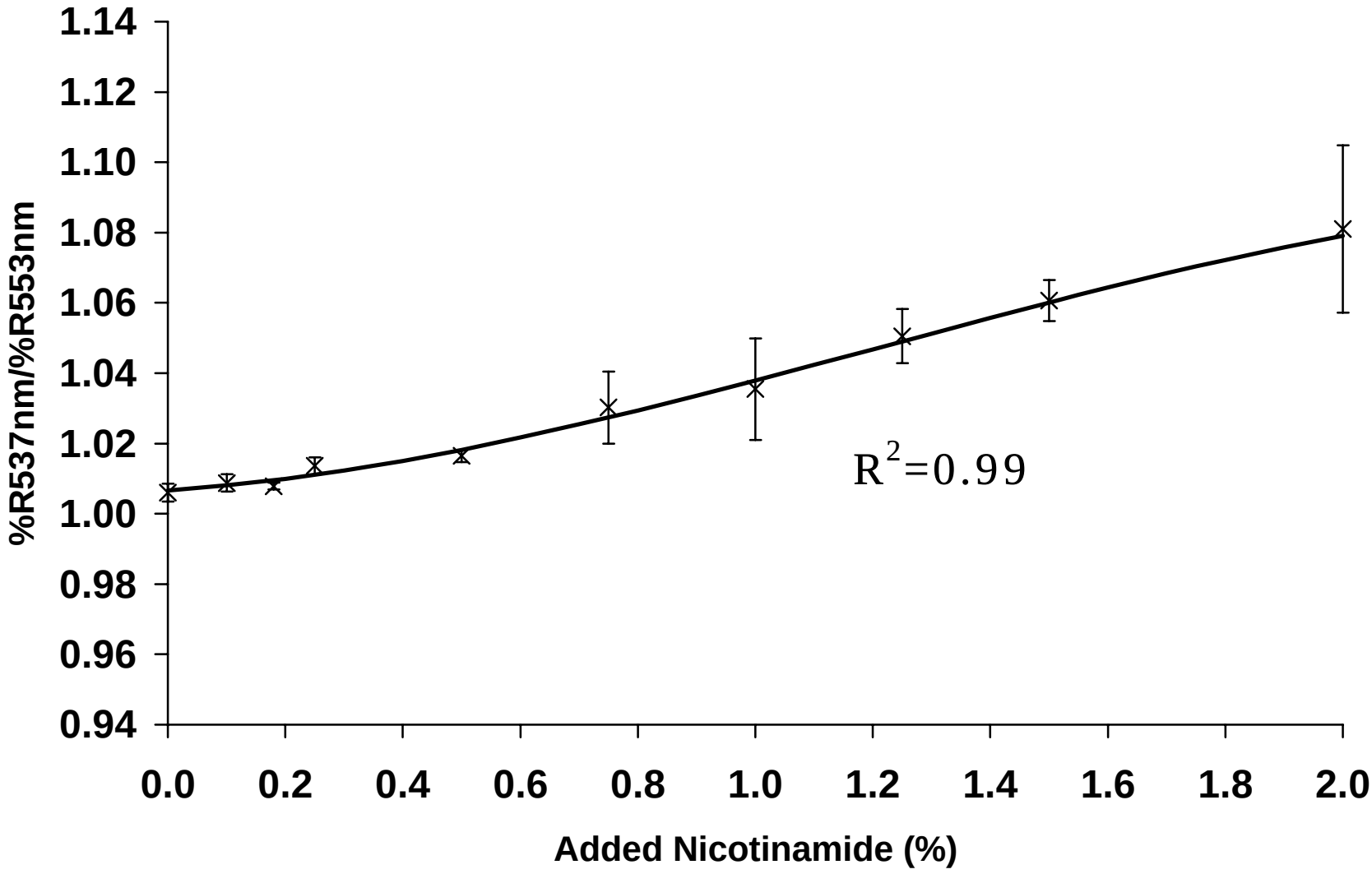

Fig. 1—Cubic model fit for %R537nm/%R553nm in samples containing added nicotinamide. Bars around means correspond to ± one S.E.

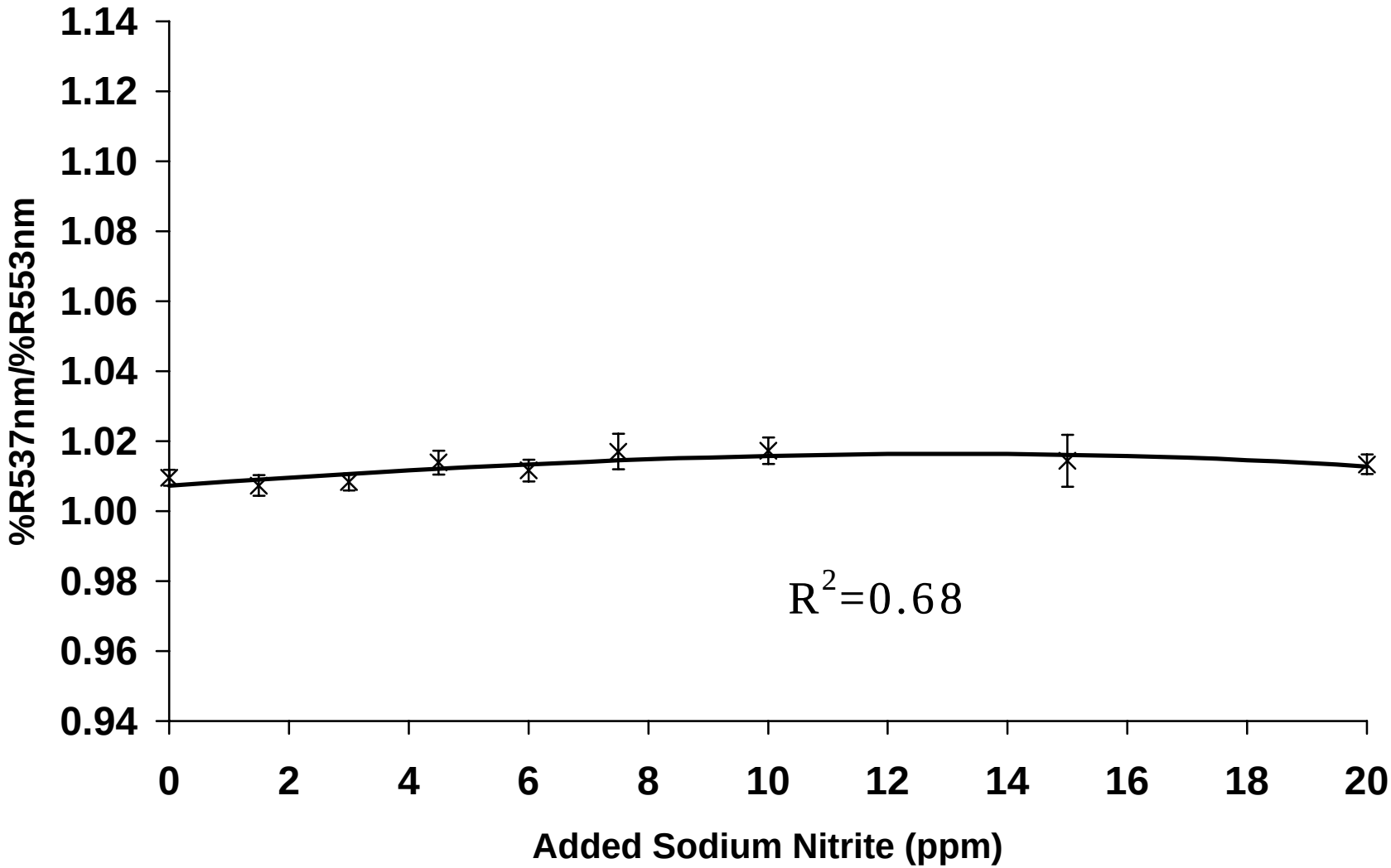

Fig. 2—Cubic model fit for %R537nm/%R553nm in samples containing added sodium nitrite. Bars around means correspond to ± one S.E.

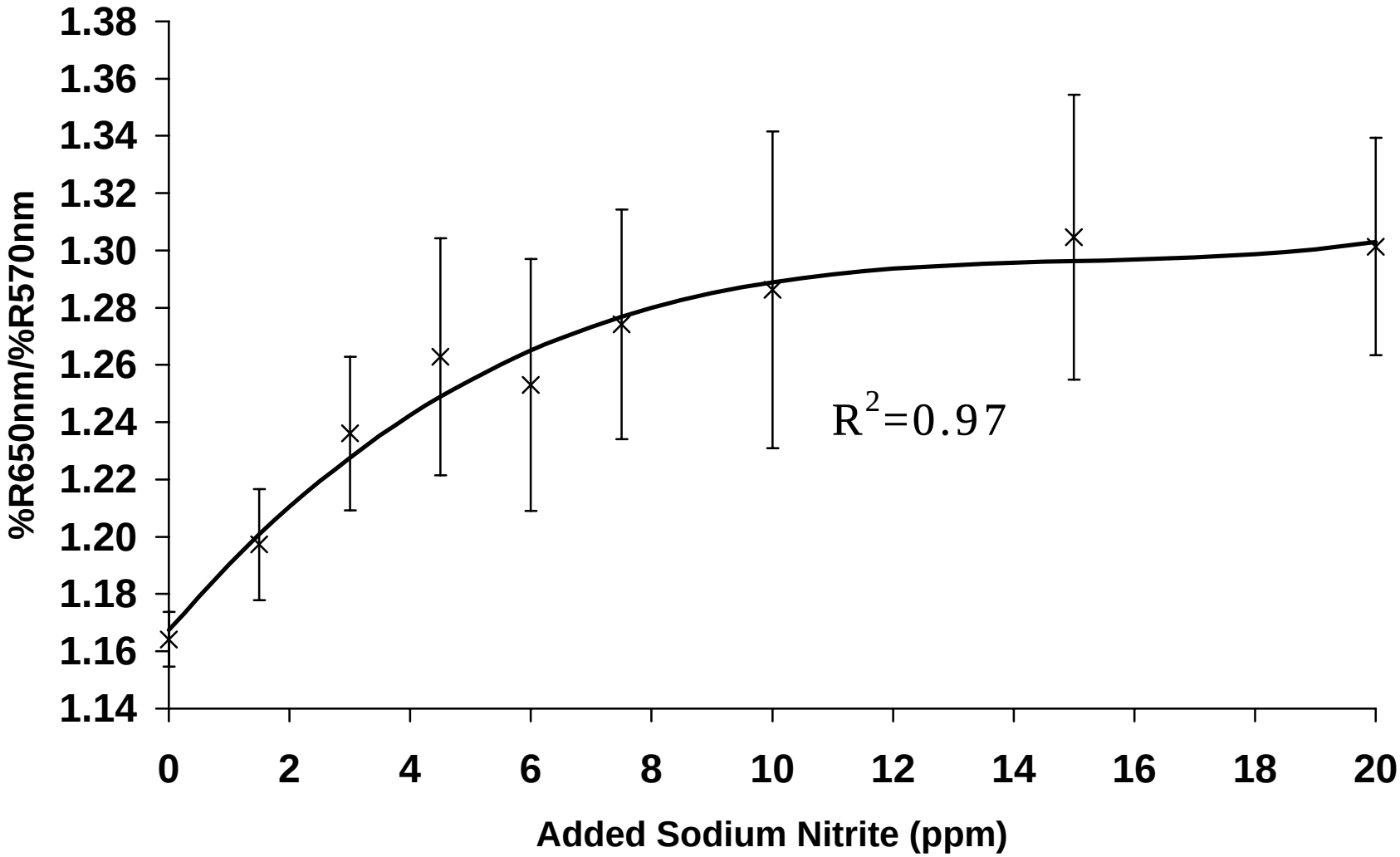

Fig. 3—Cubic model fit for %R650nm/%R570nm in samples containing added sodium nitrite. Bars around means correspond to ± one S.E.

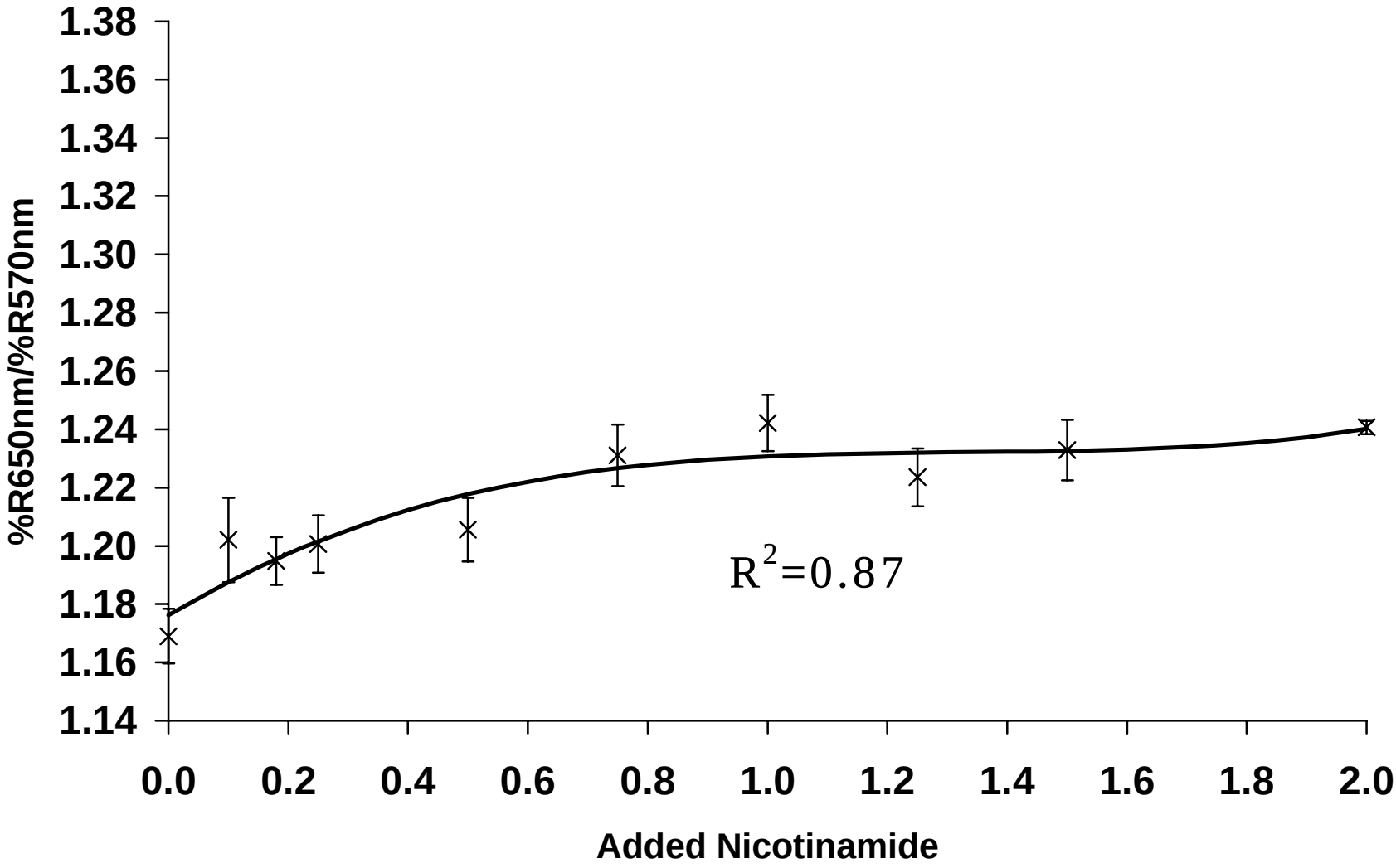

Fig. 4—Cubic model fit for %R650nm/%R570nm in samples that contain nicotinamide. Bars around means correspond to ± one S.E.

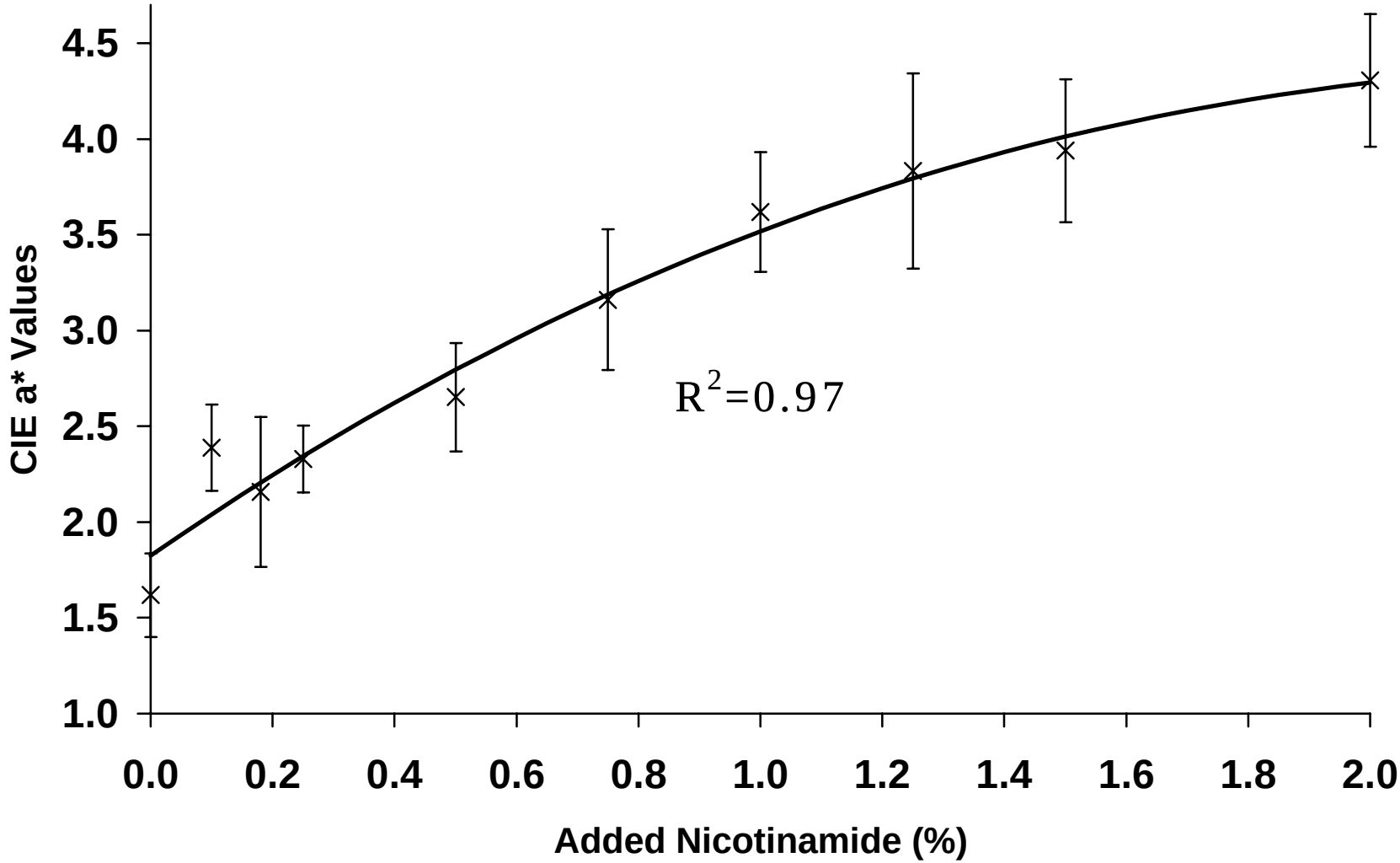

Fig. 5—Cubic model fit for CIE a* values in samples containing added nicotinamide. Bars around means correspond to ± one S.E.

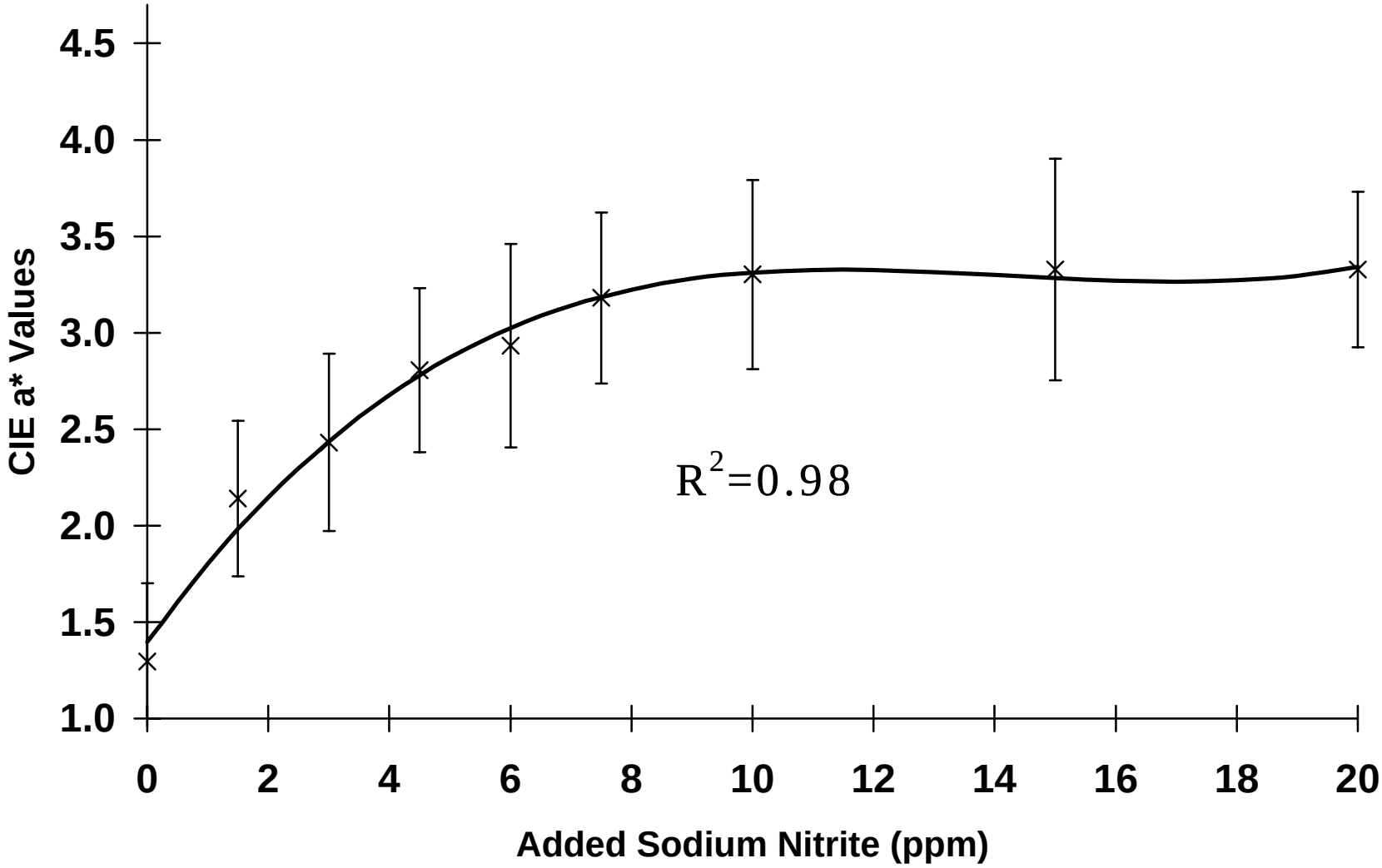

Fig. 6—Cubic model fit for CIE a* values in samples containing added sodium nitrite. Bars around means correspond to ± one S.E.

Chapter 4

Ability of Various Dairy Proteins to Reduce Pink Color Development in Cooked Ground Turkey Breast

4.1 ABSTRACT

Dairy proteins including nonfat dry milk (NFDM), two types of sodium caseinate (SC), three whey protein concentrates (WPC), and a milk protein concentrate were evaluated for their ability to reduce pink color in ground turkey samples. In samples produced to exhibit a pink color (10 ppm sodium nitrite or 1.0% nicotinamide), NFDM and one WPC reduced ($P<0.05$) CIE a* values (redness). All of the dairy proteins reduced CIE a* values in nicotinamide treated samples ($P<0.05$). However, proteins were less effective in nitrite treated samples. In samples without pink color ligand, only WPC reduced ($P<0.05$) CIE a* values, while the other proteins tested had no effect ($P<0.05$) or increased redness ($P<0.05$). All of the dairy proteins increased cooking yield ($P<0.05$). NFDM or specific WPC proteins could be incorporated into existing turkey breast formulations to reduce the pink color defect and increase yield.

4.2 INTRODUCTION

The spontaneous and unexplainable occurrence of a pink color in cooked, uncured turkey is a problem the poultry industry has faced for many years. Many causes have been blamed for this defect including nitrite or nitrate contamination (Ahn and Maurer, 1987; Froning et al., 1969; Mugler et al., 1970; Nash et al., 1985), oven gasses (Pool, 1956), heat stability of cytochrome c (Girard, 1990), and the formation of denatured globin and nicotinamide hemochromes (Cornforth et. al, 1986). Even with the large

amount of research devoted to the pink color defect, this problem is still prevalent in the industry.

Dobson and Cornforth (1992) examined the ability of nonfat dry milk (NFDM) and whey protein concentrate (WPC) to reduce pink color development of stored turkey samples. The authors based their research on reports that NFDM was able to lighten the color of bologna (Rongey and Bratzler, 1966) and that calcium casienate had a whitening effect on chicken meat (van den Hoven, 1987). NFDM was found to reduce red color development in the samples. However, WPC increased the redness (Hunter a) to a level that was "visibly pink" upon cutting. Dobson and Cornforth (1992) speculated that NFDM's ability to reduce the pink color in turkey could be related to an increase of the oxidation-reduction potential leading to a reduction in the formation of denatured hemochromes.

Schwarz et al. (1997) found that NFDM reduced red color when sodium nitrite (150 ppm), nicotinamide (1.0%) or no ligand was added. The ligands were added to the meat system in order to induce a pink color in the cooked turkey. The level of nitrite added by Schwarz et al. (1997) would not be indicative of a contamination event but rather of a purposeful addition to produce a cured product.

The objective of this research was to determine the effectiveness of seven commercially available dairy proteins on the reduction of pink color development in ground turkey breast samples treated to display a pink color. The pink color was induced in the products by the addition of 10 ppm nitrite or 1.0% nicotinamide.

4.3 MATERIALS & METHODS

4.3.1 Sample preparation

Boneless turkey breast muscles (*Pectoralis major*) were obtained from a Virginia processor for each of three replications on separate production days. The turkey breasts were transported on ice to the meat laboratory of Virginia Tech, Blacksburg, VA, where they were coarsely ground through a 1.27-cm plate (model 4532, Hobart Mfg. Co, Troy, OH). The composite turkey meat was mixed for 4 min, vacuum packaged, and frozen in barrier bags (item 030056, Koch Supplies and Equipment Co., Kansas City, MO). Frozen turkey was stored for 1 to 6 weeks at approximately –10°C. After adequate thawing at 2 to 4°C (approximately 36 to 48 h), the turkey was ground twice through a 4.76-mm plate.

The finely ground turkey samples were formulated to contain 2.0% sodium chloride, 0.5% sodium tripolyphosphate, and the pink color generating ligand treatments (no ligand, 1.0% nicotinamide, 10 ppm sodium nitrite). The above ingredients were incorporated on a meat weight basis (MWB) in a 30% solution with distilled, deionized water making up the balance of the brine solution. Also formulated on a MWB, seven commercial dairy proteins were added as a dry ingredient at 3.0%. The dairy proteins tested include nonfat dry milk (NFDM; A.C. Legg Packaging Co, Birmingham, AL), two sodium casienates (SC-A, Alanate 180; SC-B, K413, Kerry Ingredients, International, Beloit, WI), three whey protein concentrates (WPC-A, Alacen 841; WPC-B, Alacen 878; WPC-C, Alacen 882). All products with the exception of NFDM and SC-B were obtained from New Zealand Milk Products (North America) Inc., Santa Rosa, CA. As a basis of

comparison, reference samples containing no dairy proteins were also produced within the no ligand (NL-REF), nicotinamide (NIC-REF) and nitrite (NIT-REF) treated samples.

The ligand treatments, sodium nitrite (0.10 g/L), nicotinamide (100 g/L) and no ligand (distilled deionized water only) solutions (220 mL), were added to the ground turkey (2.2 kg) and mixed for 1 min (model K45SS, Kitchen Aid, St. Joseph, Michigan). Large ligand batches (2.42 kg) were split into smaller portions (275 g) for individual dairy protein treatments. Upon addition of a salt/phosphate (50 mL; 100 g/L salt and 25 g/L phosphate) solution and the dry dairy proteins (3.0%, MWB), the samples were mixed for one min using a hand mixer (model KHM3WH-1, Kitchen Aid, St. Joseph, Michigan). Approximately 45 grams of the turkey mixture was stuffed into 50 mL plastic test tubes and centrifuged (model PR-2, International Equipment Company) at 2000 x g for 10 min and stored overnight at 2 to 4°C to allow for ingredient interactions. Samples were cooked in an 85°C circulating water bath (custom built, Virginia Tech, Blacksburg, VA) to an internal temperature of 80°C then cooled to 50°C in an ice bath and stored overnight at 2 to 4°C. Internal temperature of the products was monitored throughout the cooking process using a data logger (model 5100, Electronic Controls Design, Milwaukee, Oregon) attached to thermocouple-containing samples randomly located throughout the water bath. Duplicate samples were made for each treatment combination.

4.3.2 Instrumental evaluation

Color measurements. Duplicate color measurements (CIE L*, a* and b* values) were made on the cut surface of each sample using a chroma meter (model CR-310, Minolta Corp., Osaka, Japan) with a 1-cm aperture at a right angle to the cut surface

rotating the sample 90° between measurements. The chroma meter was calibrated using a white plate (L* 97.91, a* -0.68, b* 2.45).

CIE L* is a measure of lightness in samples on a scale from 0 to 100 with high values being very light. CIE a* is a measure of the green (negative values) or red (positive values) color. CIE b* relates to the blue (negative) or yellow (positive) color. From these measurements, hue angle and chroma were calculated using the following formulas:

$$\text{Hue Angle } (h_{ab}) = \tan^{-1}\left\{\frac{b^*}{a^*}\right\}$$

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

When chroma values are similar, the hue angle is a good measure of the red color. Values near 90° relate to yellow color whereas smaller values (closer to 0°) relate to very red samples. Chroma is a measure of the color saturation.

Pigment determination. Two spectrophotometric reflectance measurements were taken on the freshly cut surface using a UV/visible scanning spectrophotometer (model 2101PC, Shimadzu Inc., Kyoto, Japan) from 400 nm to 700 nm. The instrument was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm and fast scan speed and was calibrated using a white calibration plate (L* 97.91, a* -0.68, b* 2.45).

The reflectance (R) ratio of % R at 537 nm divided by % R at 553 nm was used to predict NICHEME in the no ligand and nicotinamide treated samples (Schwarz et al., 1998b). NITHEME was determined in the no ligand and nitrite containing samples by the reflectance ratio of %R at 650 nm divided by %R at 570 nm (Erdman and Watts, 1957; Kraft and Ayres, 1954; AMSA, 1991).

pH determination. The pH of raw samples was measured using a pH electrode (item 13-620-298, Fisher Scientific, Pittsburgh, PA) and a pH meter (model AR25, Fisher Scientific, Pittsburgh, PA) by diluting 15-g samples with 150 mL of distilled water. The diluted meat samples were homogenized (20 mm shaft, part number 225318, Virtis Co., Inc., Gardiner, NY) for 20 s prior to pH meausrement.

4.3.3 Yield determination

The cooking yield was determined by the following formula:

$$\text{Cooking Yield (\%)} = \left(1 - \frac{\text{raw product weight - cooked product weight}}{\text{raw product weight}}\right) \times 100$$

4.3.4 Statistical analysis

Statistical analysis was performed using the one-way analysis of variance procedure prepared by SAS (1990) to identify statistical differences between dairy protein treatments within ligand treatments. Differences among means for the responses of yield, pH, and color measurements was determined using the Tukey's Least Significant Difference procedure.

4.4 RESULTS & DISCUSSION

4.4.1 Yield and pH

All of the dairy protein treatments increased yield ($P<0.05$) compared to the reference samples (no added dairy protein) within each of the ligand treatments (Table 1). The increase in yield can be attributed to the ability of the dairy proteins to bind water, increase gelation, and stabilize the meat system during cooking (Huffman, 1996; van den

Hoven, 1987). On average, yield increased approximately 3.7, 2.5, and 3.6% for the no ligand, nicotinamide, and nitrite treated samples, respectively, compared to the reference samples within each ligand group. Although not the intent of the research, it was unknown why the nicotinamide treated samples had a higher overall yield than the other ligand treatments.

Although there were some differences ($P<0.05$) among pH of the treatments, these differences were considered marginal in terms of practical significance (data not shown). The mean pH values ranged from only 6.17 to 6.25 for all of the ligand treatments.

4.4.2 Color measurements

CIE a* values (redness). When no pink color generating ligand was added, NFDM had no effect on CIE a* values ($P>0.05$) as compared to the NL-REF (Fig. 1), which disagrees with the work of Dobson and Cornforth (1992) and Schwarz et al. (1998a) who found NFDM to reduce redness in samples where no pinking agents were added. The natural variation in raw materials may have obscured NFDM's ability to reduce redness. In addition, the percentage of brine solution added for both of the studies mentioned was only 10 to 20% as compared to the 30% added in this work.

Compared to the NL-REF, WPC-A did not affect CIE a* values ($P>0.05$), whereas WPC-B, SC-A, SC-B and MPC increased CIE a* ($P<0.05$) and WPC-C decreased CIE a* ($P<0.05$). These observations are very important because they demonstrate that the addition of certain dairy proteins may increase the redness in commercial products, while others may reduce redness. Additionally, the increase in redness is not consistent within the specific classes of dairy proteins, as was demonstrated

among the WPC proteins. Dobson and Cornforth (1992) found that the WPC they tested increased the redness of the products to a "visibly pink" color with a corresponding increase in Hunter a (redness) values. In our work, WPC-B increased the redness ($P<0.05$) as was reported by Dobson and Cornforth (1992). Since, WPC-A had no effect on CIE a* values and WPC-C reduced CIE a* values ($P<0.05$), these proteins could be added to increase yield and possibly improve texture without an increase in redness.

Nicotinamide, which has been reported to increase CIE a* values (Cornforth et al., 1986; Claus et al., 1994), also visually increased the NIC-REF samples as compared to the NL-REF samples. All of the dairy proteins tested reduced the nicotinamide induced pink ($P<0.05$; Fig. 1) as compared to the NL-REF. NFDM, all WPCs and MPC reduced values well below the no protein control ($P<0.05$) virtually eliminating the pink color induced by the nicotinamide (Fig. 1). While the mechanism for the pink color reduction has not been identified, it could be speculated that an interaction between the nicotinamide and dairy protein or the dairy protein and the heme during the cooking process might be involved.

Addition of 10 ppm sodium nitrite also visually increased the red color of the turkey samples compared to the NL-REF. Overall, the dairy proteins were not as effective at reducing nitrite-induced pinking compared to the NIC samples as was reported in Schwarz et al. (1998a). NFDM and WPC-C were the only two proteins able to reduce ($P<0.05$) the pink color among the nitrite treated samples compared to the NIT-REF (Fig. 1). SC-A, SC-B, WPC-B and MPC increased the redness ($P<0.05$) when nitrite was added (Fig.1).

When evaluating the dairy proteins ability to reduce pink color in turkey under all three ligand treatments, NFDM, WPC-A and WPC-C were the only proteins tested that consistently reduced or had no effect on the redness.

CIE b* (yellowness). When no pink generating ligand was added, NFDM, SC-A and WPC-A did not affect the yellowness (CIE b*) compared to the NL-NP control ($P>0.05$; Fig. 2). However, SC-B, WPC-B and MPC reduced CIE b* ($P<0.05$), while WPC-C was the only protein to increase the yellowness ($P<0.05$).

Within the nicotinamide treated samples, NFDM, all of the WPC proteins and the MPC increased the yellowness ($P<0.05$) compared to the NIC-REF samples. Neither SC protein tested had an effect on the yellowness ($P>0.05$) compared to the NIC-REF.

Overall, the nitrite samples appeared to be less yellow than the other ligand treatments. Within the NIT samples, only WPC-C increased the yellowness ($P<0.05$) as compared to the NL-NIT control. SC-B, WPC-B and MPC reduced CIE b* ($P<0.05$) while NFDM, SC-A, WPC-A had no effect ($P>0.05$).

CIE L* (lightness). Increases in the lightness of turkey breast products may improve the overall appearance of the product. NFDM and all WPCs increased the lightness of the turkey samples regardless of ligand treatment ($P<0.05$; Table 1), in contrast to Dobson and Cornforth (1992), where no increase was found. Both of the SC proteins tested reduced the lightness of the samples ($P<0.05$) in all three ligand treatments with only one exception where the reduction in L* by SC-B was not significant ($P>0.05$). MPC increased lightness in nicotinamide treated samples ($P<0.05$) but did not have an effect in no ligand and nitrite treated samples ($P>0.05$).

Chroma and hue angle. Chroma, a measure of color saturation, does not relate to specific hue differences among samples. Within the ligand and dairy protein treatments, differences in chroma measurements were minimal (Table 1). Because the chroma values were similar, the hue offers a comparison of red color among turkey samples with lower values tending to be redder in color. Among the experimental samples, the hue angle measurements varied from a high of 91.9, which was yellow in color to a low value of 47.0, which was visibly pink in color (Table 1). Similar to CIE a* and CIE b* values, the dairy proteins did not consistently change the hue angle values over the different ligand treatments.

Generally, the hue angle measurements (Table 1) followed the same trends as the CIE a* value measurements (Fig. 1). The hue angle of the no ligand added and nitrite treated samples followed the same trends as the CIE a* values when compared to the appropriate reference samples, with only a few exceptions. In both cases NFDM and WPC-A did not have any effect ($P>0.05$) on hue angle (Table 1). SC-B, WPC-B and MPC decreased the hue angle ($P<0.05$), and thus reduced yellowness and increased redness. SC-A caused a reduction in hue angle when no ligand was added ($P<0.05$), but had no effect in the nitrite treated samples. Only WPC-C was able to increase the hue angle as compared to the reference controls (NL-REF and NIT-REF). Within the nicotinamide treated samples, all the dairy proteins reduced the hue angle ($P<0.05$).

4.4.3 Pigment measurement

In the nicotinamide treated samples, the NICHEME was reduced ($P<0.05$) by all of the dairy proteins with the exception of the two SC proteins (Table 1). With the exception of the two SC protein, reductions in pigment followed similar patterns to the

reduction of red color (CIE a*). Because the SC proteins reduced redness but did not reduce NICHEME, it is possible that SC-A and SC-B may have a different mode of action in reducing the nicotinamide-induced redness.

NFDM, WPC-A and WPC-C reduced NITHEME whereas SC-B, WPC-B and MPC increased NITHEME. These changes in NICHEME also followed the same trend as reductions and increases in CIE a* values. This agrees with work done by Ahn and Maurer (1989a) where they found increased redness as NITHEME increased.

4.5 CONCLUSIONS

NFDM, WPC-A and WPC-C could be added to processed turkey products to potentially increase product yield, increase lightness, and reduce pink color generation caused by variations in nicotinamide content or nitrite contamination. Processors need to be aware that addition of some dairy proteins at a 3% level can increase pink color in turkey products. Further research should focus on the mechanism(s) responsible for the reduction in pink color by specific dairy proteins. An opportunity exists for a dairy protein or some component of dairy proteins to reduce or eliminate the pink color defect. WPC-C and NFDM both show the best potential for this effect.

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Table 1- Color, pigment, yield, and pH measurements in samples containing no dairy protein, nonfat dry milk (NFDM), sodium caseinates (SC-A and B), whey protein concentrates (WPC-A, B and C) and milk protein concentrate (MPC) with 1.0% nicotinamide, 10 ppm sodium nitrite and no ligand added.

	Dairy	Yield	Color Measurements			Pigment Prediction	
Ligand	Protein[g]	(%)	CIE L*	Hue angle	Chroma	NICHEME[h]	NITHEME[i]
No Ligand	None	92.92[c]	78.39[c]	75.84[b]	9.51[b]	1.005[c]	1.184[c]
	NFDM	96.63[a]	79.31[b]	76.27[b]	9.45[b]	1.007[c]	1.151[d]
	SC-A	94.97[b]	76.65[d]	72.08[c]	9.41[b]	1.016[c]	1.203[b]
	SC-B	97.26[a]	77.19[d]	63.75[d]	8.80[c]	1.062[a]	1.231[a]
	WPC-A	97.25[a]	80.10[a]	78.01[b]	9.49[b]	1.002[c]	1.147[d]
	WPC-B	96.87[a]	79.30[b]	60.99[e]	8.90[c]	1.060[a]	1.231[a]
	WPC-C	97.14[a]	80.01[a]	85.03[a]	10.17[a]	0.985[d]	1.129[e]
	MPC	96.48[a]	78.50[c]	70.52[c]	8.85[c]	1.040[b]	1.198[bc]
	LSD	1.01	0.599	0.048	0.372	0.0141	0.0163
Nicotinamide	None	96.14[d]	77.34[d]	66.93[f]	8.70[bc]	1.018[a]	-
	NFDM	98.69[ab]	79.44[abc]	86.55[bc]	9.86[a]	0.986[c]	-
	SC-A	97.57[c]	75.67[e]	71.77[e]	8.53[c]	1.013[ab]	-
	SC-B	98.14[bc]	76.50[de]	73.95[e]	8.68[bc]	1.022[a]	-
	WPC-A	99.24[a]	80.02[a]	88.88[ab]	10.01[a]	0.990[c]	-
	WPC-B	98.77[ab]	78.87[bc]	80.08[d]	8.97[b]	1.003[b]	-
	WPC-C	98.84[ab]	79.71[ab]	84.90[c]	9.91[a]	0.988[c]	-
	MPC	98.85[ab]	78.52[c]	91.94[a]	9.89[a]	0.990[c]	-
	LSD	0.73	0.938	0.059	0.341	0.0105	
Nitrite	None	93.29[c]	78.01[d]	62.33[bc]	8.46[bc]	-	1.318[d]
	NFDM	97.46[a]	78.96[c]	67.08[ab]	8.87[ab]	-	1.271[e]
	SC-A	94.88[b]	75.89[e]	57.78[c]	8.32[bcd]	-	1.350[cd]
	SC-B	97.01[a]	76.26[e]	46.95[d]	7.76[de]	-	1.451[a]
	WPC-A	97.14[a]	79.55[ab]	64.22[b]	8.57[abc]	-	1.262[e]
	WPC-B	96.88[a]	78.82[c]	51.23[d]	8.10[cde]	-	1.366[bc]
	WPC-C	97.43[a]	79.86[a]	71.15[a]	9.18[a]	-	1.209[f]
	MPC	97.05[a]	77.37[d]	51.05[d]	7.64[e]	-	1.395[b]
	LSD	0.92	0.731	4.819	0.664		0.0368

[a-f] Means in the same column and ligand treatment with different superscripts are different (P<0.05)
[g] Dairy proteins added at 3.0% on a meat weight basis
[h] Nicotinamide hemochrome (NICHEME, %R 537nm/%R 553 nm), higher values indicates more NICHEME
[l] Nitrosylhemochrome (NITHEME, %R 650 nm/%R 570 nm), higher values indicates more NITHEME

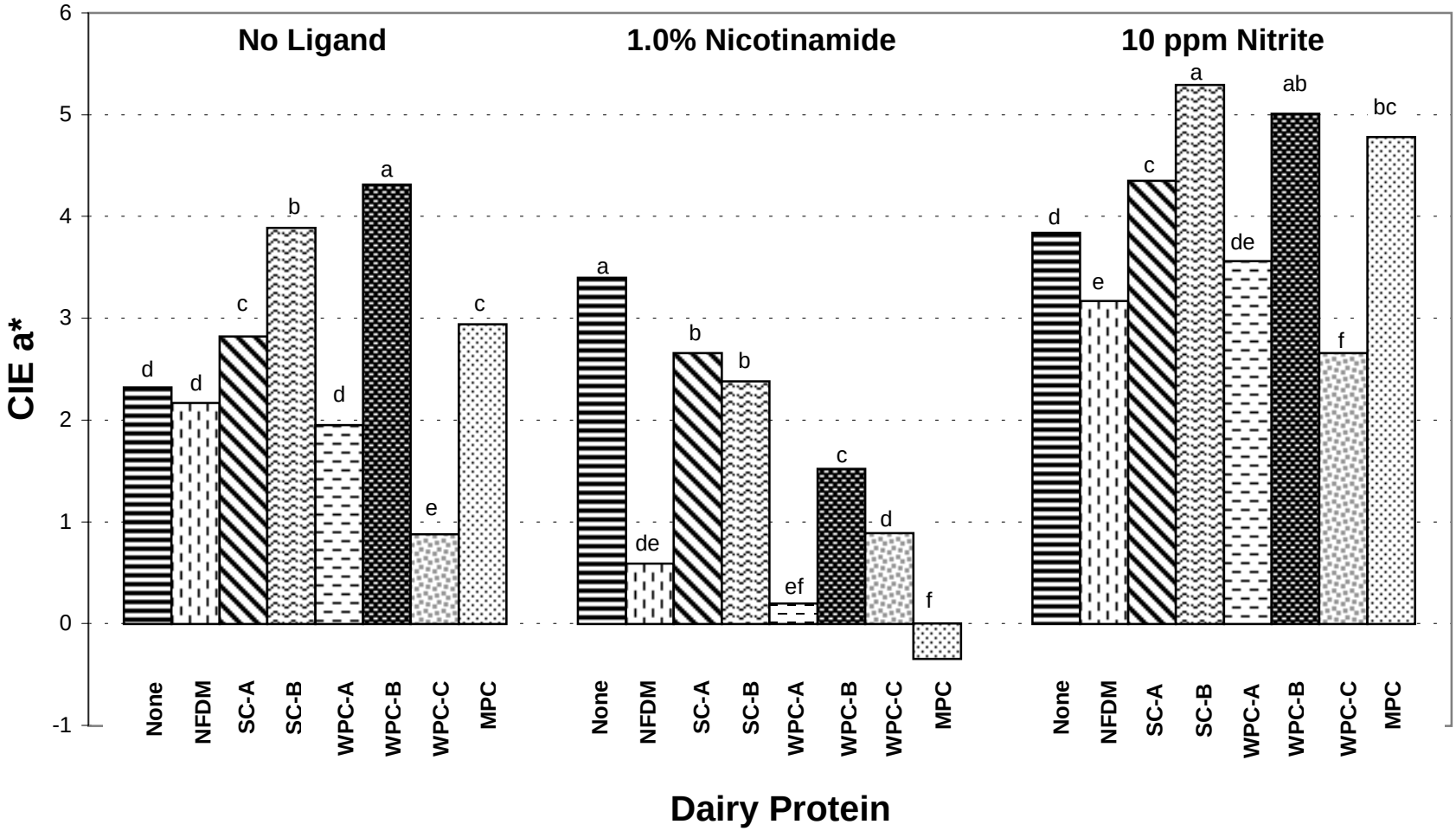

Fig 1 - CIE a* measurements of samples containing dairy proteins (3.0% based on meat weight) with no ligand, nicotinamide (1.0%), and sodium nitrite (10 ppm). Dairy proteins tested included nonfat dry milk (NFDM), sodium caseinate (SC-A and SC-B), whey protein concentrate (WPC-A, WPC-B, and WPC-C) and milk protein concentrate (MPC). Means within ligand groups with unlike letters are different (P<0.05).

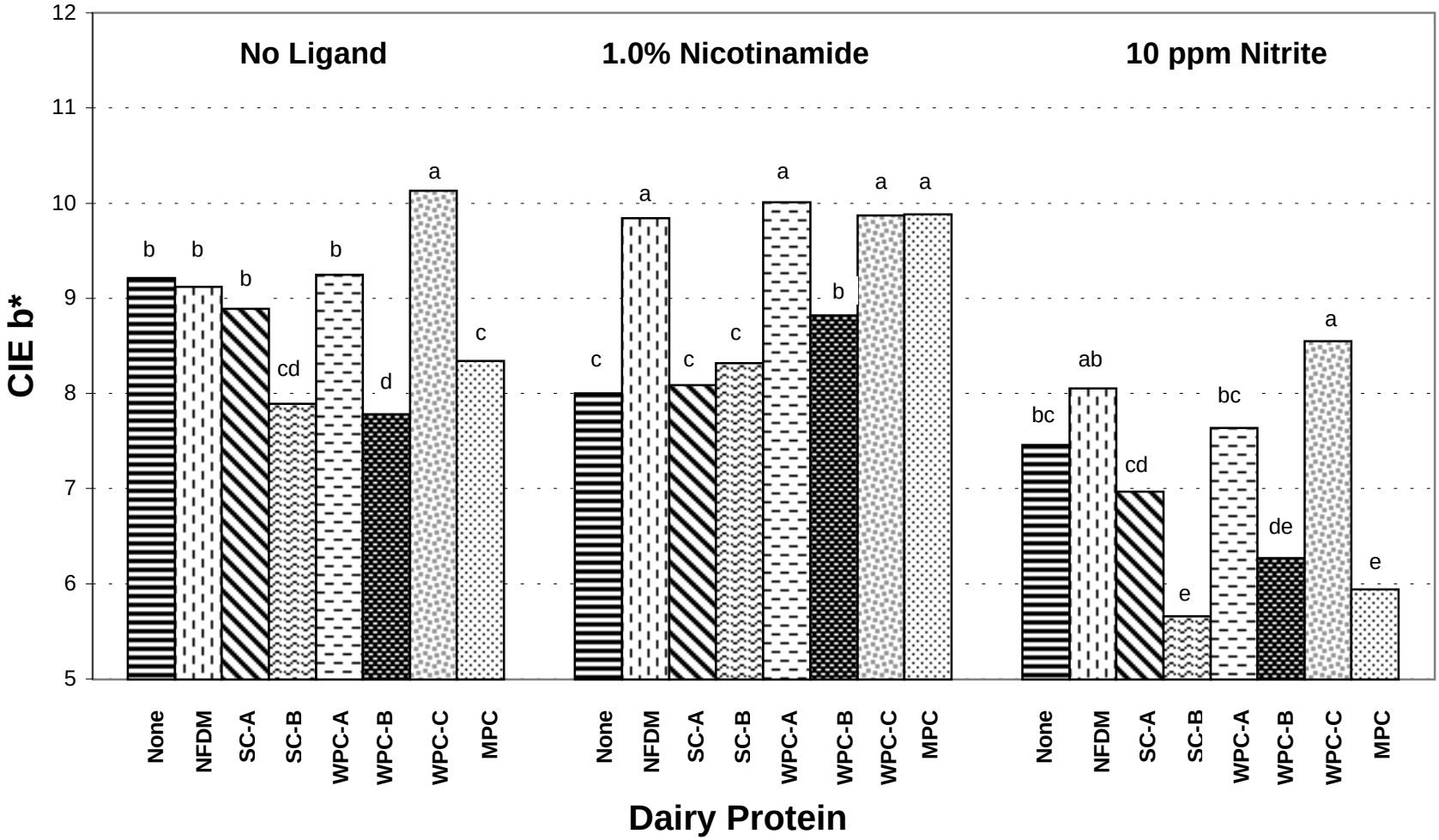

Fig 2 - CIE b* measurements of samples containing 3.0% of various dairy proteins with no ligand, nicotinamide (1.0%), sodium nitrite (10 ppm). Dairy proteins tested included nonfat dry milk (NFDM), sodium caseinate (SC-A and SC-B), whey protein concentrate (WPC-A, WPC-B, and WPC-C) and milk protein concentrate (MPC). Means within ligand groups with unlike letters are different (P<0.05).

Chapter 5

Reduction of Pink Color Development in Cooked, Uncured, Ground Turkey Breast by the Addition of Dairy Proteins

5.1 ABSTRACT

Nonfat dry milk (NFDM), sodium caseinate (SC), whey protein concentrate (WPC), and combinations of each were evaluated for abilities to reduce pink color development in cooked, ground, uncured turkey breast. Protein treatments were also evaluated in the presence of pink color generating ligands (nicotinamide, 1%, sodium nitrite, 10 ppm, and sodium nitrate, 50 ppm) with and without EDTA (200 ppm). NFDM and WPC at levels as low as 1.5% were effective in reducing CIE a* values ($P<0.05$) regardless of ligand treatment; SC was not. EDTA reduced pink color within all protein and ligand treatments. Poultry producers can reduce pink color development in further processed products by selective addition of dairy proteins.

5.2 INTRODUCTION

A sporadic pink color development in cooked, uncured turkey products remains a problem within the poultry industry. A great deal of research has been devoted to identifying the mechanisms responsible for this defect. Prevention methods have focused on the reduction of the presence of nitrites and/or nitrates in the meat (Ahn and Maurer, 1987; Nash et al., 1985; Froning et al., 1969). This method focuses on the prevention of nitrosylhemochrome (NITHEME) formation. NITHEME, the heat stable pink pigment of cured meat products, can also form in turkey at very low concentrations of nitrite, with as

little as 1 ppm nitrite increasing red color (Ahn and Maurer, 1987). However, other pigments have also been identified as causing the pink color defect.

Cornforth et al. (1986) attributed the sporadic pink color development in turkey to hemochrome complexes. Nicotinamide is found in turkey at relatively high concentrations and can form complexes with the heme containing pigments. Cornforth et al. (1986) speculated that nicotinamide hemochrome (NICHEME) and denatured globin hemochrome were responsible for the heat stable pink color. In addition, the authors found that reflectance patterns from commercially pink samples were similar to the reflectance patterns of experimental samples containing nicotinamide. Reducing conditions within the meat block were reported to be important in the formation of the hemochrome-complexes.

Regardless of the pigments responsible for the pink color formation, a method to consistently reduce the formation of these pink pigments would benefit the poultry industry. Dobson and Cornforth (1992) reported that in samples that were not induced to display a pink color defect, the addition of nonfat dry milk (NFDM) reduced the pink color of turkey breast meat. Schwarz et al. (1997) reported that 3.0% added NFDM reduced the pink color associated with the addition of both nicotinamide (1.0%) and sodium nitrite (150 ppm) in ground turkey products. Schwarz et al. (1998a) found similar results in whole muscle products when NFDM was added at 2% in nicotinamide treated samples. However, 2.0% NFDM was unable to reduce nitrite (20 ppm or 150 ppm) induced pink color. It is possible that the difference was related to the lower level of NFDM used in the latter study, or that the NFDM was less dispersed in the whole muscle product as compared to the ground product.

The objective of this research was to compare the effect of NFDM, WPC, and SC on the development of pink color in cooked, ground turkey breast. The proteins were evaluated in samples not treated to induce a pink color, and those treated to induce pink color (1.0% nicotinamide, 10 ppm sodium nitrite and 50 ppm sodium nitrate) with and without EDTA (0 and 200 ppm) added.

5.3 MATERIALS & METHODS

Boneless, turkey breast muscles (*Pectoralis major*) were obtained from a Virginia processor for the three replications on three separate production days. The breasts were transported on ice to the meat laboratory of Virginia Tech. Upon arrival, the meat was stored on ice for 12 to 24 h. The breasts for each replication were ground through a 1.27-cm plate (model 4532, Hobart Manufacturing Co., Troy, Ohio), and mixed for 4 min. The composite sample was then separated into eight barrier bags (item 030056, Koch Supplies and Equipment Co., Kansas City, MO) and stored at -10°C for one to four weeks. Four portions of meat were thawed at 2 to 4°C in covered containers for 36 to 48 h for each production day. Prior to final formulation and mixing, the turkey was removed from the vacuum bags and ground twice through a 4.76-mm plate.

A basic formulation was used to produce a comminuted turkey product consisting of finely ground turkey breast, 2.0% sodium chloride (NaCl), and 0.5% sodium tripolyphosphate (STP), and if added, a pink generating ligand (1.0% nicotinamide, 10 ppm sodium nitrite, or 50 ppm sodium nitrate). The above non-meat ingredients were added on a meat weight basis (MWB) in a 30% MWB brine solution.

Based on a mixture model simplex lattice design using statistical response surface methods (Myers and Montgomery, 1995), eleven dairy protein treatments were performed on four ligand treatments (no ligand, nicotinamide, sodium nitrite, and sodium nitrate). The protein treatments consisted of NFDM (A.C. Legg Packaging Co., Birmingham, AL), sodium caseinate (SC; Alanate 180, New Zealand Milk Products, (North America) Inc, Santa Rosa, CA), and whey protein concentrate (WPC; Alacen 882, New Zealand Milk Products (North America)) individually at 1.5 and 3.0%. The proteins were also tested in combination with each other in equal portions (1.5% each, MWB) of two proteins (NFDM and SC, NFDM and WPC, and SC and WPC) and equal proportions (0.75% each, MWB) of all three proteins. The same treatments were also performed in the presence of 200 ppm EDTA.

To provide the proper final concentrations, the ligands were added to large meat batches (7 kg) in solutions (700 mL) containing 100 g/L nicotinamide, 0.100 g/L sodium nitrite, 0.500 g/L sodium nitrate and if necessary the EDTA. After addition of the ligand solutions, the meat was mixed for 2 min (model A-200, Hobart Manufacturing Co., Troy, Ohio). The large batches were split into smaller portions (550 g), to which 100 mL of a salt/phosphate (100 g/L NaCl and 25 g/L STP) and the dairy proteins were added. After addition, the components were mixed 45 s using a hand mixer (model KHM3WH-1, Kitchen Aid, St. Joseph, MI). The mixtures were stuffed into six-50 mL plastic test tubes and centrifuged (model PR-2, International Equipment Company, Boston, MA) at 2000 x g for 10 min. Samples were stored overnight to allow for ingredient interactions and then cooked to 74 and 80°C (3 tubes for each endpoint temperature) in a 85°C circulating water bath (custom built, Virginia Tech, Blacksburg, VA). Internal temperature of the

products was monitored using a data logger (model 5100, Electronic Controls Design, Milwaukee, Oregon) attached to several thermocouple containing samples which were randomly placed throughout the water bath. Upon completion of the cooking process, tubes were cooled to 50°C in an ice bath then stored overnight at 2 to 4°C. The three sample tubes cooked to each endpoint temperature were again split into the storage times of 1, 20 and 40 d.

The storage samples (20 and 40 d) were removed from the tubes, weighed for yield information and vacuum packaged in barrier bags (item 030026, Koch Supplies and Equipment Co., Inc., Kansas City, MO). Samples were stored in the dark (1.4°C) and the temperature was monitored throughout the storage time using a data logger (model D200, Dickson Co., Addison, IL).

5.3.1 Nitrite and nitrate determination for raw turkey

Since each of the three replication was prepared from a composite of the different breasts, representative frozen samples from each replication were analyzed for nitrite and nitrate (AOAC, 1990). Analysis was performed by Warren Analytical Laboratory (Greenley, CO).

5.3.2 pH determination

The pH of both composite samples for each replication and each of the treatments were measured using a pH electrode (item 13-620-298, Fisher Scientific, Pittsburgh, PA) and a pH meter (model AR25, Fisher Scientific, Pittsburgh, PA) by diluting 15-g samples with 150 mL of distilled water. The diluted meat samples were homogenized for 20 s (20 mm shaft, part number 225318, Virtis Co., Inc., Gardiner, NY) prior to pH measurement.

5.3.3 Cooking yield determination

The cooking yield was determined by the following formula:

$$\text{Cooking Yield (\%)} = \left(1 - \frac{\text{raw product weight - cooked product weight}}{\text{raw product weight}}\right) \times 100$$

5.3.4 Color measurements

Immediately after cutting duplicate samples, CIE L*, a*, and b* measurements were made using a chroma meter (model CR-310, Minolta Corp., Osaka, Japan) with a 1-cm aperture at a right angle to the cut surface. Samples were rotated 90° between measurements. The device was calibrated using a white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44). Two readings were taken on each sample, one at time 0 after cutting and one at 2 min after cutting. The 2-min samples were exposed to fluorescent light (model F20T12/30U, Phillips Lighting Co., Somerset, NJ) at an intensity of 1076 lux.

5.3.5 Pigment determination

Two reflectance spectrophotometric measurements were made with a UV/visible scanning spectrophotometer (model 2101PC, Shimadzu Inc., Kyoto, Japan) from 400 to 700 nm. The instrument was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm, fast scan speed and calibrated a white calibration plate (CIE L* = 97.91, a* = -0.71, b* = +2.44). From the reflectance data, NICHEME was measured using the reflectance (R) ratio of %R at 537 divided by %R at 553 nm (Schwarz et al., 1998b). NITHEME was measured by the ratio %R at 650 nm divided by %R at 570 nm (Erdman and Watts, 1957; Kraft and Ayres, 1954; AMSA, 1991).

5.3.6 Statistical analysis

The experimental design used was a second order response surface mixture model allowing for interaction of the NFDM, SC, and WPC and a non-linear response relative to factor levels. A simplex lattice design with all combinations restricted to less than or equal to 3% added protein was used to explore factor levels between 0 and 3% for the three proteins of interest. Statistical analysis was performed using SAS (1990). The response surface design allowed not only for testing of differences in means at various levels, but also allowed for a surface of all the responses to be constructed. Once the surface has been determined, the response at any combination of explanatory values can be estimated.

5.4 RESULTS & DISCUSSION

5.4.1 Composite turkey analysis

Both nitrate and nitrite were determined to be less than 2 ppm for all three replications, indicating that the natural level of these components remained constant. Ahn and Maurer (1987) found the turkey used in their work to vary from 0 to 0.7 ppm nitrite and 3.8 to 21 ppm nitrate.

5.4.2 Effect of dairy proteins on pH and yield

Overall, the dairy proteins had very minimal effect on the pH of the samples. In samples not treated with a pink color generating ligand or EDTA, no differences ($P<0.05$) in pH were found (Table 1). The addition of dairy proteins did affect the cooking yields of the various products (Table 1). Within the samples cooked to 74°C, WPC at 1.5 and

3.0% was the only protein to statistically increase yield ($P<0.05$) when used alone. The combination of WPC with SC also increased yield. Although not statistically compared, samples cooked to 80°C generally had lower yields than the 74°C samples (Table 1). With the exception of SC at either 1.5% or 3.0% alone, all of the dairy proteins and combinations of proteins increased yield ($P<0.05$) when cooked to 80°C.

5.4.3 Effects of dairy proteins on CIE a* values

The main objective of this research was to determine the dairy proteins' abilities to reduce pink color in the samples, and therefore the results focused on CIE a* values and pigment measurements. Unless otherwise noted, comparison for color and pigment measurement will be made based on the no protein containing samples within the same ligand treatments.

Samples prepared with 50 ppm sodium nitrate did not appear to differ in redness from the samples where no pink color ligand was added. Because sodium nitrate addition did not appear affect color, results will not be discussed but have been included in some of the data tables for completeness. The lack of color development in the nitrate treated samples was likely the result of inadequate time for the reduction of nitrate, to nitrite and then to nitric oxide. Nash et al. (1985) reported that a pink color formed in turkey after 3 d of contact with 50 ppm nitrate containing ice water.

The main effects including protein, air and light exposure (fade), storage time, and endpoint temperatures and all two- and three-way interactions were evaluated for CIE a* values. Overall the protein combinations and storage time had highly significant effects on CIE a* values regardless of ligand treatment (Table 2). End point temperature did have a significant overall effect on no ligand and nitrite containing samples. A two-

way interaction for protein x storage time was also significant (Table 2) for no ligand (Fig.1), nicotinamide (Fig. 2) and for nitrite (Fig. 3) containing samples. Upon further analysis, the interaction was significant due to slight variations in the individual proteins' abilities to limit increases in CIE a* values over the 40 d storage time (Figure 1 through 3). These variations, were slight and most likely of little practical importance.

Pink color reduction in samples containing no pink generating ligand. When added at 3.0%, all of the dairy proteins, NFDM, SC and WPC reduced ($P<0.05$) CIE a* values (Table 3). NFDM and WPC caused approximately a 57% reduction, while SC only reduced CIE a* values by 9.7%. In addition, all of the combinations of two (1.5% of each protein) or three (0.75% of each protein) proteins reduced ($P<0.05$) CIE a* values (Table 3).

NFDM (1.5%) or WPC (1.5%) reduced CIE a* values ($P<0.05$) while SC (1.5%) did not have an effect (Table 3; $P>0.05$). While the reduction of CIE a* values by NFDM and WPC at 1.5% was not a great as the 3.0% protein containing samples, addition of only 1.5% was adequate at reducing pink color. This information could help producers determine the level of specific dairy proteins to add to their products.

The results of the samples containing no added ligand agree with those of Dobson and Cornforth (1992), who found that 3.0% NFDM reduced redness and Schwarz et al. (1998a), who reported that 2.0% NFDM reduced CIE a* values. However, the reduction of CIE a* values by the addition of WPC-C disagrees with the results of Dobson and Cornforth (1992), who reported an increase in redness with the addition of WPC.

Pink color reduction in samples containing nicotinamide and sodium nitrite. While not compared statistically, nicotinamide and sodium nitrite substantially increased

the CIE a* values of the no protein control samples (Table 3). Overall, the dairy proteins' effects on CIE a* values were similar in both nicotinamide and nitrite containing samples. NFDM and WPC at 3.0% reduced ($P<0.05$) the nicotinamide induced pink color by approximately 65 and 72%, respectively, and nitrite induced pink color by 28.9 and 47% (Table 3). A reduction of this magnitude would most likely result in no visible pink color, especially in the nicotinamide samples. From the results presented, it can also be noted that the dairy proteins were less effective at reducing nitrite rather than nicotinamide induced pink. NFDM and WPC at 1.5% also reduced CIE a*. SC at a level of 3.0% or 1.5% did not effect CIE a* values (Table 3; $P>0.05$).

The effect of endpoint temperature and storage time on CIE a*. Of the ligand treatments, only nicotinamide samples had no overall change in CIE a* values ($P>0.05$) between the 74 and 80° endpoint temperatures (Table 4). In all other ligand treatments, increased endpoint temperature caused an overall increase ($P<0.05$) in CIE a* values. In all cases, CIE a* values increased ($P<0.05$) over storage time (Table 4). This agrees with the work done by Claus et al. (1994) who found an increase in CIE a* values over storage time in samples treated with nicotinamide.

The effect of EDTA on CIE a*. The addition of EDTA dramatically reduced CIE a*, virtually eliminating all red color over all of the treatments tested including the protein, end-point temperature, and storage (Tables 2 and 3). Therefore, discussion of the dairy proteins' effects are not necessary. Although EDTA is not approved for use in meat products, its ability to reduce redness in products may be the result of competitive binding of myoglobin. With EDTA bound to the heme ring, other potential ligands may

be prevented from interacting with the heme, thus reducing the formation of pink pigments.

5.4.4 Effects of dairy proteins on pigment measurements

Similar to the results of CIE a*, protein and storage time were highly significant in the nicotinamide, nitrite and nitrate treated samples (Table 2). The effect of endpoint temperature was only significant among the nitrite treated samples. The only two-way interaction that was significant was protein x storage time. No three-way interactions were significant.

Among the nitrite treated samples (Table 5), the general trends in NITHEME content followed the CIE a* measurements, suggesting that the reduction in redness is related to a reduction in NITHEME. To further support the apparent connection between the pigment measurements and CIE a*, the NIHEME measurement also increased over the 40 d storage time as did the CIE a* values (Table 6).

Among the nicotinamide treated samples, NICHEME content did not clearly follow the same trends as CIE a*. It is possible that the NICHEME measurement is obscured by the addition of dairy proteins or that the reduction in pink color is not related to a reduction of NICHEME. Further work using multiple methods of pigment determination should be done to clearly find the dairy proteins' effects on these pigments.

The effect of EDTA on the pigment measurements. Among the nicotinamide and nitrite treated samples, no differences were found among the dairy protein treatments as compared to the no protein containing reference samples (Table 5).

5.4.5 Response surface analysis

A typical set of conditions were chosen to exhibit the results of the response surface analysis and to aid in identifying combinations of dairy proteins that may be effective at reducing the red color (CIE a*) of the turkey products. For the analysis, data from samples that were cooked to 74°C, stored for 20 days, and contained no EDTA were used to evaluate the ability of protein combinations to reduce CIE a* for the no ligand, nicotinamide, and nitrite treated samples. The benefit of the response surface design over standard multiple comparison methods results in the fact that prediction of CIE a* can be done over a wide range dairy protein treatments, not just the specific combinations that were tested. Analysis of this type of data can be done by setting a "target" for the response being evaluated. In this case, lower CIE a* values would be more desirable for turkey product, because the objective of the experimentation was to reduce pink color by the addition of dairy proteins.

The response surface contour plots (Figs. 4 through 6) display how the predicted CIE a* values respond across all combinations of NFDM, SC, and WPC with total percentages of 0% up to and including 3%. Within the three-dimensional space represented, cross-sectional contour plots display the predicted CIE a* values. Within each contour plot, horizontal lines signify that one of the dairy proteins did not significantly influence CIE a* values. Diagonal or curved lines suggest an interaction between two or three proteins.

In samples where no ligand was added, both NFDM and WPC reduced CIE a* values, while SC had very little effect (Fig. 4). The highest values of CIE a* (approximately 3.0) were found where no protein was added (bottom left corner of the

plot). As NFDM was increased toward 3%, CIE a* values dropped to approximately 1.2. Likewise, as WPC was increased CIE a* values dropped to approximately 1.1.

Nicotinamide treated samples responded similarly to the no added ligand samples (Fig. 5). As expected, the CIE a* values were higher than the no added ligand samples when no dairy protein was added. NFDM and WPC again reduced CIE a* values as their concentrations increased. WPC appeared to have the largest reduction effect on CIE a* values.

When the samples were treated with nitrite (Fig. 6) CIE a* values where no dairy protein were added to the turkey formulations were between the no ligand added and nicotinamide treated levels. Thus indicating that nicotinamide increased redness more than the nitrite. However, the dairy proteins were less able to reduce redness over the range of levels tested. Unlike the no ligand and the nicotinamide samples, the lowest predicted CIE a* values occurred at a combination of dairy proteins not actually tested, where the approximate levels were NFDM = 1.0, WPC = 1.5, SC = 0.5.

5.5 CONCLUSIONS

The addition of specific dairy proteins can be useful in reducing the pink color in turkey breast products. NFDM and WPC were able to reduce the pink color associated with both nitrite and nicotinamide, and also reduced redness in samples that were not treated with any pink color generating ligand. The addition of 200 ppm EDTA, while not approved for use in meat products at any level, was able to eliminate the pink color regardless of ligand treatment. If in the future, EDTA is approved for meat products, it may have a substantial role in eliminating the pink color defect. However, currently, the dairy proteins NFDM and WPC may be the best alternative.

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Table 1 – Mean pH and Cooking Yields for ground turkey samples containing various dairy proteins without EDTA or pink color generating ligand added.

Protein (%)	Protein[d]	pH	Cooking Yield (%) Endpoint Temperature 74°C	80°C
0	No Protein	6.07	90.19[c]	86.82[c]
3	NFDM	6.12	94.79[abc]	93.19[ab]
	SC	6.08	92.70[abc]	89.91[bc]
	WPC	6.07	96.34[a]	94.63[a]
1.5	NFDM	6.04	94.34[abc]	92.21[ab]
	SC	6.04	91.25[bc]	89.16[bc]
	WPC	6.06	95.46[ab]	93.05[ab]
1.5 (each)	NFDM + SC	6.09	94.50[abc]	92.71[ab]
	NFDM + WPC	6.10	94.74[abc]	93.46[ab]
	SC + WPC	6.08	95.81[ab]	93.70[ab]
0.75 (each)	NFDM + SC + WPC	6.07	94.80[abc]	92.93[ab]
	LSD	0.10	4.78	4.70

[abc] Means within a column with different letters are different (P<0.05).

[d] Dairy proteins tested include nonfat dry milk (NFDM; 1.5 and 3%), sodium caseinate (SC; 1.5% and 3%) and whey protein concentrate (WPC; 1.5 and 3%), and all combinations of two proteins at 1.5% (3% total) and combination of all three at 0.75%.

Table 2 – Significance of main effects and two-way interactions for CIE a* values and pigment measurements in ground turkey samples containing dairy proteins.

Independent	CIE a * Values				Pigment		
Variable	No Ligand	NIC[b]	Nitrite	Nitrate	NIC[b]	Nitrite[b]	Nitrate[b]
				NO EDTA			
Protein	****	****	****	****	****	****	****
Fade		**					*
Storage time	****	****	****	****	****	****	****
EPT	**		****	*		****	
Protein x Fade							
Protein x Storage time	****	****	**	****	*	*	****
Protein x EPT							
Fade x Storage time							
Fade x EPT							
Storage time x EPT							
				200 ppm EDTA			
Protein	****	****	****	****	**		****
Fade		*					
Storage time	****	****	****	****	****	****	****
EPT	****	****	**	****	**		*
Protein x Fade							
Protein x Storage time		****					
Protein x EPT				*			*
Fade x Storage time							
Fade x EPT							
Storage time x EPT	**				**		

[a] Independent variable significance, **** <0.0001, ***<0.001, **<0.01, and *<0.05; Protein = dairy proteins tested include nonfat dry milk, sodium caseinate, and whey protein concentrate: FADE= 2 minute air and light exposure; Storage time = 1, 20 , or 40 d; EPT = end point temperature (74 or 80°C)

[b] In nicotinamide (NIC) treated samples, the reflectance ratio of %R at 537 nm divided by %R at 553 nm was used to predict nicotinamide hemochrome; in sodium nitrite and sodium nitrate treated samples, the ratio of %R at 650 nm divided by %R at 570 nm was used to predict nitrosylhemochrome.

Table 3 – CIE a* values for turkey samples containing dairy proteins in the presence of no pink color generating ligand, nicotinamide (1.0%), nitrite (10 ppm), and nitrate (50 ppm), with and without 200 ppm EDTA present.

Protein (%)	Protein[g]	No Ligand	Ligand		
			Nicotinamide	Nitrite	Nitrate
		NO EDTA			
0	No Protein	3.10^{a}	4.96^{a}	4.53^{a}	3.12^{a}
3	NFDM	1.29^{e}	1.75^{def}	3.22^{b}	1.33^{e}
	SC	2.80^{b}	4.62^{a}	4.17^{a}	2.65^{b}
	WPC	1.25^{e}	1.37^{f}	2.40^{e}	1.32^{e}
1.5	NFDM	1.89^{c}	2.78^{b}	3.32^{b}	1.93^{c}
	SC	2.88^{ab}	4.63^{a}	4.34^{a}	2.88^{ab}
	WPC	1.72^{cd}	2.43^{bc}	2.96^{bcd}	1.84^{c}
1.5 (each)	NFDM + SC	1.68^{cd}	2.42^{bc}	3.11^{bc}	1.53^{de}
	NFDM + WPC	1.28^{e}	1.61^{ef}	2.62^{de}	1.32^{e}
	SC + WPC	1.47^{de}	1.95^{cde}	2.75^{cde}	1.48^{de}
0.75 (each)	NFDM + SC + WPC	1.76^{cd}	2.32^{bcd}	2.96^{bcd}	1.78^{cd}
	LSD	0.293	0.579	0.411	0.307
		200 ppm EDTA			
0	No Protein	0.77^{bcd}	1.45^{ab}	1.82^{abc}	0.89^{abcd}
3	NFDM	0.84^{abc}	1.10^{bcd}	2.13^{a}	0.98^{ab}
	SC	1.10^{a}	1.70^{a}	1.60^{bc}	0.88^{abcd}
	WPC	0.60^{cd}	0.60^{d}	1.64^{bc}	0.64^{e}
1.5	NFDM	0.91^{ab}	1.76^{a}	2.09^{a}	1.11^{a}
	SC	0.74^{bcd}	1.62^{ab}	1.56^{c}	0.93^{abcd}
	WPC	0.54^{d}	1.29^{abc}	1.94^{ab}	0.72^{cde}
1.5 (each)	NFDM + SC	0.71^{bcd}	1.80^{a}	1.92^{abc}	0.94^{abc}
	NFDM + WPC	0.62^{cd}	0.74^{cd}	1.91^{abc}	0.70^{de}
	SC + WPC	0.50^{d}	1.28^{abc}	1.60^{bc}	0.78^{bcde}
0.75 (each)	NFDM + SC + WPC	0.74^{bcd}	1.42^{ab}	1.92^{abc}	0.82^{bcde}
	LSD	0.289	0.591	0.381	0.238

[a-f] Means within a column and EDTA treatment group (none vs. 200 ppm) with unlike superscript letters are different (P<0.05). Means were pooled over end-point cooking temperature, storage time, and air and light exposure.

[g] Dairy proteins tested include nonfat dry milk (NFDM), sodium caseinate (SC) and whey protein concentrate (WPC).

Table 4 – CIE a* values at two endpoint cooking temperatures and three storage times with and without the presence of 200 ppm EDTA.

			Ligand		
		No Ligand	Nicotinamide	Nitrite	Nitrate
		NO EDTA			
End Point Temperature (C)	74	1.86[b]	2.77	3.15[b]	1.88[b]
	80	1.98[a]	2.84	3.46[a]	1.97[a]
	LSD	0.076	0.150	0.106	0.079
Storage Time (d)	1	1.54[c]	1.91[c]	2.65[c]	1.50[c]
	20	1.92[b]	2.99[b]	3.41[b]	1.93[b]
	40	2.30[a]	3.51[a]	3.87[a]	2.35[a]
	LSD	0.111	0.220	0.156	0.117
		200 ppm EDTA			
End Point Temperature (C)	74	0.63[b]	1.11[b]	1.75[b]	0.74[b]
	80	0.84[a]	1.57[a]	1.91[a]	0.96[a]
	LSD	0.075	0.153	0.099	0.062
Storage Time (d)	1	0.48[c]	0.44[c]	1.56[c]	0.56[c]
	20	0.74[b]	1.45[b]	1.78[b]	0.86[b]
	40	0.98[a]	2.13[a]	2.15[a]	1.14[a]
	LSD	0.110	0.224	0.145	0.090

[a-c] Means within a column, EDTA treatment group (none vs. 200 ppm), and end point temperature or storage time with unlike superscript letters are different ($P<0.05$). Means for end-point temperature were pooled over air and light exposure, storage time and dairy proteins. Means for storage time were pooled over air and light exposure, end-point temperature, and dairy proteins

Table 5 – Pigment values for turkey samples containing dairy proteins in the presence of no pink color generating ligand, nicotinamide (1.0%), nitrite (10 ppm), and nitrate (50 ppm), with and without 200 ppm EDTA present.

				Ligand Added		
		No Added Ligand		Nicotinamide	Nitrite	Nitrate
				Pigment Predictor[f]		
(%)	Protein[g]	537/553	650/570	537/553	650/570	650/570
				NO EDTA		
0	No Protein	0.976	1.243^{a}	1.006^{abc}	1.349^{a}	1.285^{a}
3	NFDM	0.979	1.166^{e}	0.988^{d}	1.264^{b}	1.184^{cd}
	SC	0.978	1.239^{a}	1.009^{a}	1.346^{a}	1.269^{a}
	WPC	0.979	1.157^{e}	0.985^{d}	1.208^{d}	1.162^{e}
1.5	NFDM	0.979	1.187^{bc}	0.996^{abcd}	1.271^{b}	1.200^{bc}
	SC	0.976	1.234^{a}	1.008^{ab}	1.355^{a}	1.275^{a}
	WPC	0.978	1.173^{bcde}	0.990^{d}	1.244^{bc}	1.204^{b}
1.5 (each)	NFDM + SC	0.979	1.183^{bcd}	0.995^{bcd}	1.265^{b}	1.189^{bcd}
	NFDM + WPC	0.980	1.159^{e}	0.987^{d}	1.228^{cd}	1.175^{de}
	SC + WPC	0.979	1.172^{cde}	0.989^{d}	1.240^{bc}	1.189^{bcd}
0.75 (each)	NFDM + SC + WPC	0.980	1.189^{b}	0.993^{cd}	1.241^{bc}	1.194^{bc}
	LSD	0.005	0.016	0.014	0.032	0.017
				200 ppm EDTA		
0	No Protein	0.984^{ab}	1.159^{ab}	0.997^{ab}	1.199	1.155^{abc}
3	NFDM	0.985^{a}	1.150^{bcd}	0.997^{ab}	1.208	1.151^{abcde}
	SC	0.982^{b}	1.172^{a}	1.002^{ab}	1.206	1.167^{a}
	WPC	0.986^{a}	1.142^{cd}	0.994^{b}	1.193	1.137^{e}
1.5	NFDM	0.986^{a}	1.154^{bcd}	1.005^{a}	1.208	1.158^{abc}
	SC	0.984^{ab}	1.163^{a}	0.998^{ab}	1.200	1.163^{ab}
	WPC	0.985^{ab}	1.141^{cd}	1.000^{ab}	1.204	1.145^{cde}
1.5 (each)	NFDM + SC	0.984^{ab}	1.155^{bc}	1.002^{ab}	1.200	1.156^{abc}
	NFDM + WPC	0.986^{a}	1.139^{d}	0.994^{b}	1.196	1.138^{de}
	SC + WPC	0.987^{a}	1.147^{bcd}	1.002^{ab}	1.191	1.148^{bcde}
0.75 (each)	NFDM + SC + WPC	0.986^{a}	1.153^{bcd}	1.002^{ab}	1.202	1.153^{abcd}
	LSD	0.003	0.015	0.010	0.028	0.016

[a-e] Means within a column and EDTA treatment group (none vs. 200 ppm) with unlike superscript letters are different (P<0.05). Means were pooled over end-point cooking temperature, storage time, and air and light exposure.

[f] The reflectance ratio of %R at 537 nm divided by %R at 553 nm was used to predict nicotinamide hemochrome; The reflectance ratio of %R at 650 nm divided by %R at 570 nm was used to predict nitrosylhemochrome.

[g] Dairy proteins tested include nonfat dry milk (NFDM), sodium caseinate (SC) and whey protein concentrate (WPC).

Table 6 – Pigment measurements at two endpoint cooking temperatures and three storage times with and without the presence of 200 ppm EDTA.

		No Ligand Added		Ligand Added		
				Nicotinamide	Nitrite	Nitrate
		Pigment Predictor[d]				
		537/553	650/570	537/553	650/570	650/570
		NO EDTA				
End Point Temperature (C)	**74**	0.979[a]	1.188[a]	0.995[a]	1.264[b]	1.191[a]
	80	0.977[b]	1.189[a]	0.995[a]	1.284[a]	1.190[a]
	LSD	0.001	0.004	0.004	0.008	0.004
Storage Time (d)	**1**	0.974[b]	1.155[c]	0.978[b]	1.212[c]	1.130[c]
	20	0.979[a]	1.192[b]	1.005[a]	1.285[b]	1.157[b]
	40	0.980[a]	1.221[a]	1.002[a]	1.324[a]	1.176[a]
	LSD	0.002	0.006	0.005	0.012	0.006
		200 ppm EDTA				
End Point Temperature (C)	**74**	0.985[a]	1.148[a]	0.998[b]	1.199[a]	1.149[b]
	80	0.984[a]	1.148[a]	1.001[a]	1.202[a]	1.154[a]
	LSD	0.001	0.004	0.003	0.007	0.004
Storage Time (d)	**1**	0.987[a]	1.128[c]	0.991[b]	1.175[c]	1.130[c]
	20	0.984[b]	1.147[b]	1.003[a]	1.195[b]	1.152[b]
	40	0.981[c]	1.169[a]	1.004[a]	1.232[a]	1.173[a]
	LSD	0.001	0.006	0.004	0.011	0.006

[a-c] Means within a column, EDTA treatment group (none vs. 200 ppm), and end point temperature or storage time with unlike superscript letters are different (P<0.05). Means for end-point temperature were pooled over air and light exposure, storage time and dairy proteins. Means for storage time were pooled over air and light exposure, end-point temperature, and dairy protein.

[d] The reflectance ratio of %R at 537 nm divided by %R at 553 nm was used to predict nicotinamide hemochrome; The reflectance ratio of %R at 650 nm divided by %R at 570 nm was used to predict nitrosylhemochrome.

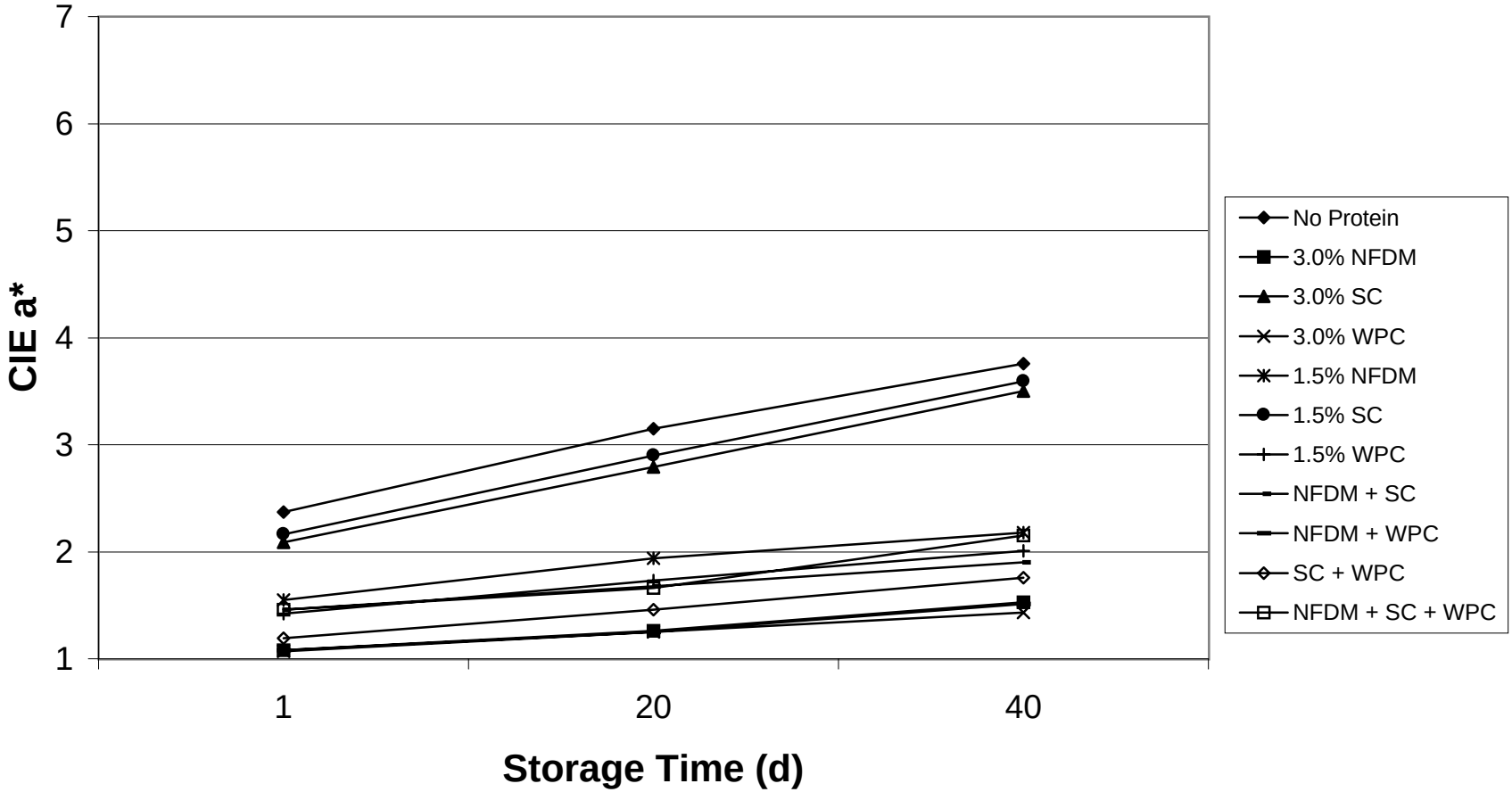

Fig. 1–Protein x storage time Interaction effect of CIE a* in samples containing no ligand

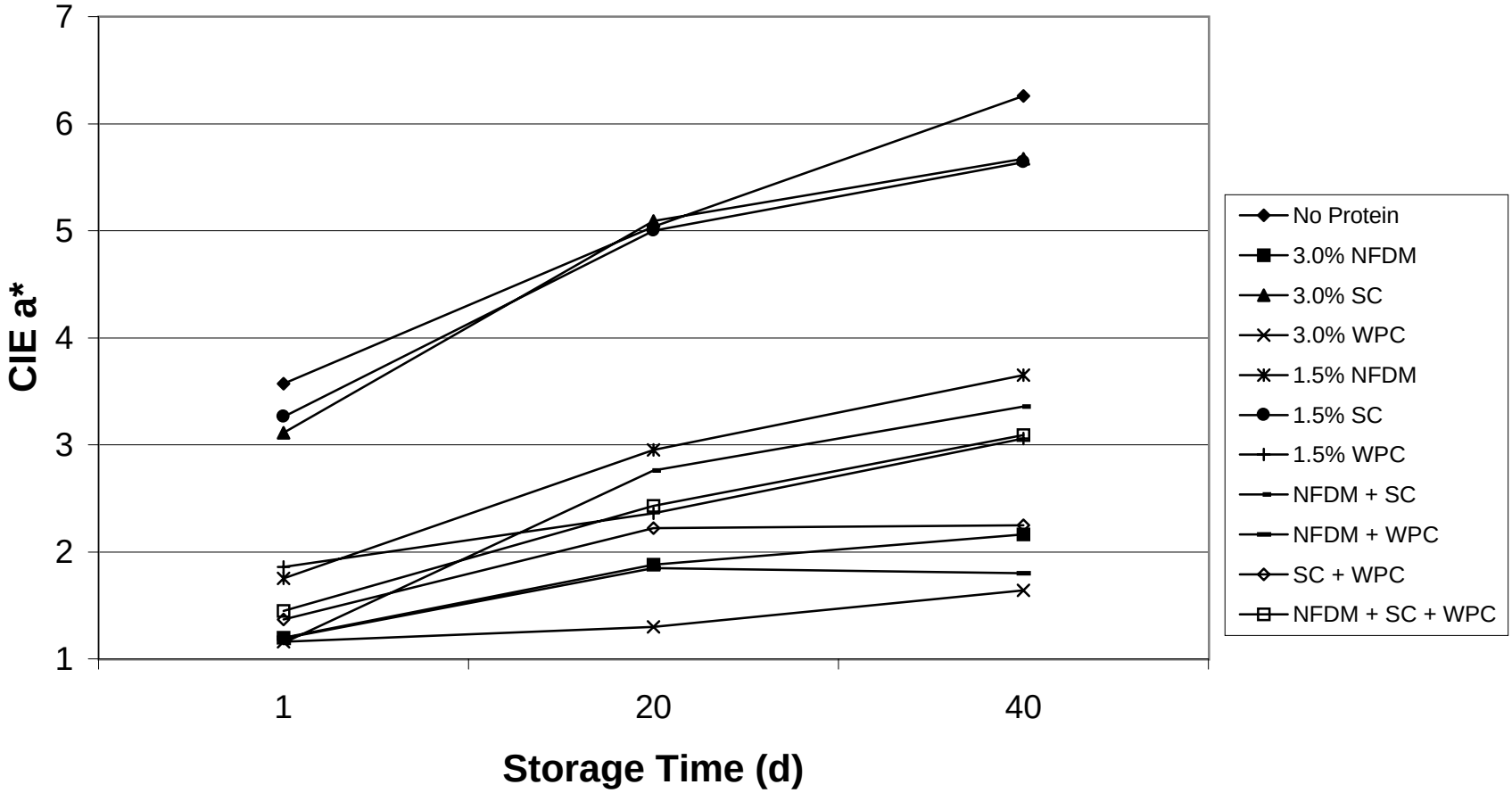

Fig. 2–Protein x storage time Interaction effect of CIE a* in samples containing nicotinamide

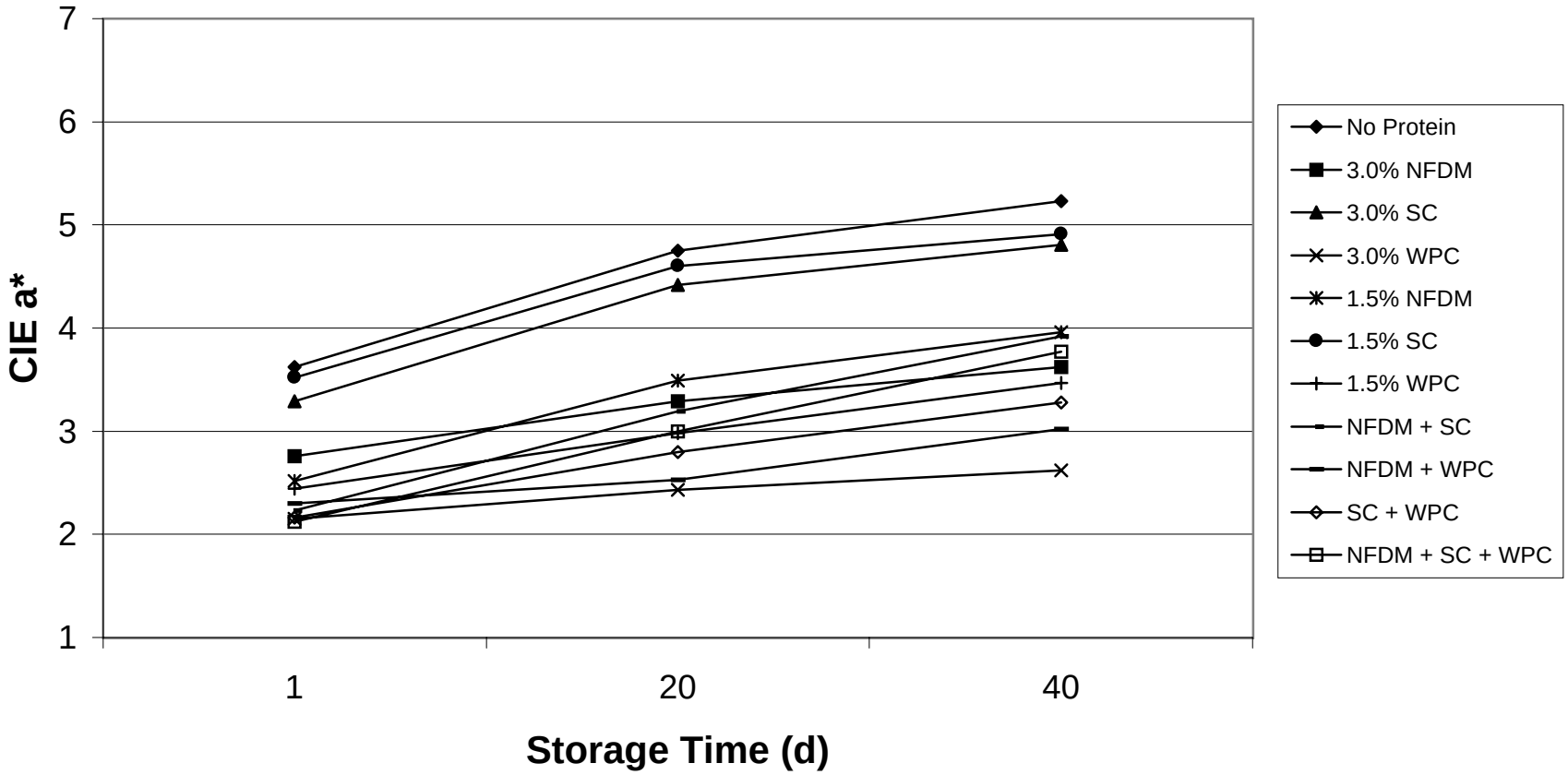

Fig. 3–Protein x storage time interaction effect of CIE a* in samples containing sodium nitrite

NO PINK COLOR GENERATING LIGAND ADDED

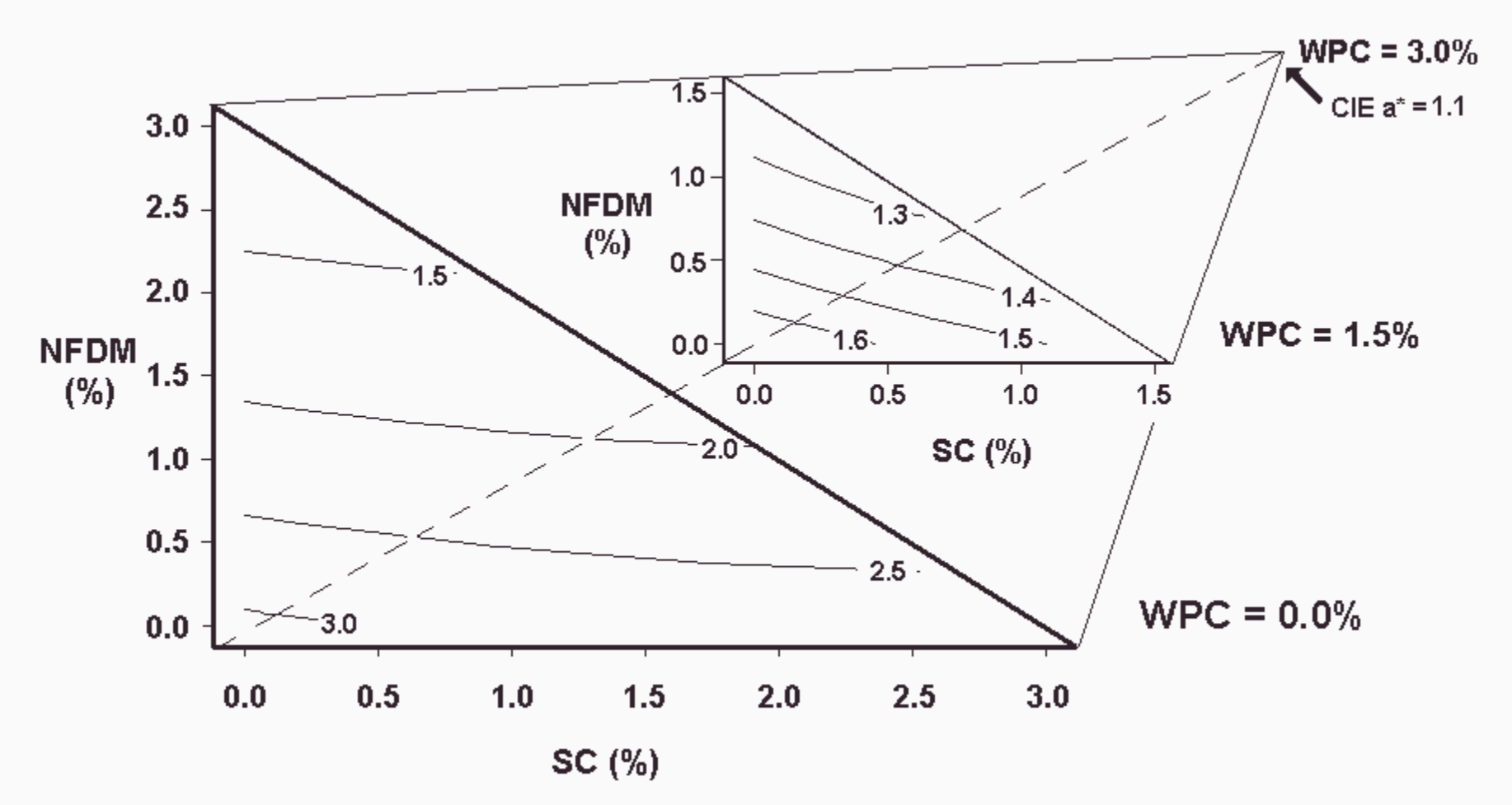

Fig. 4–Contour plots of CIE a* (redness) for samples containing the dairy proteins nonfat dry milk (NFDM), sodium caseinate (SC), and whey protein concentrate (WPC) alone, or in combinations of up to 3.0% added protein with no pink color generating ligand added.

1.0% NICOTINAMIDE

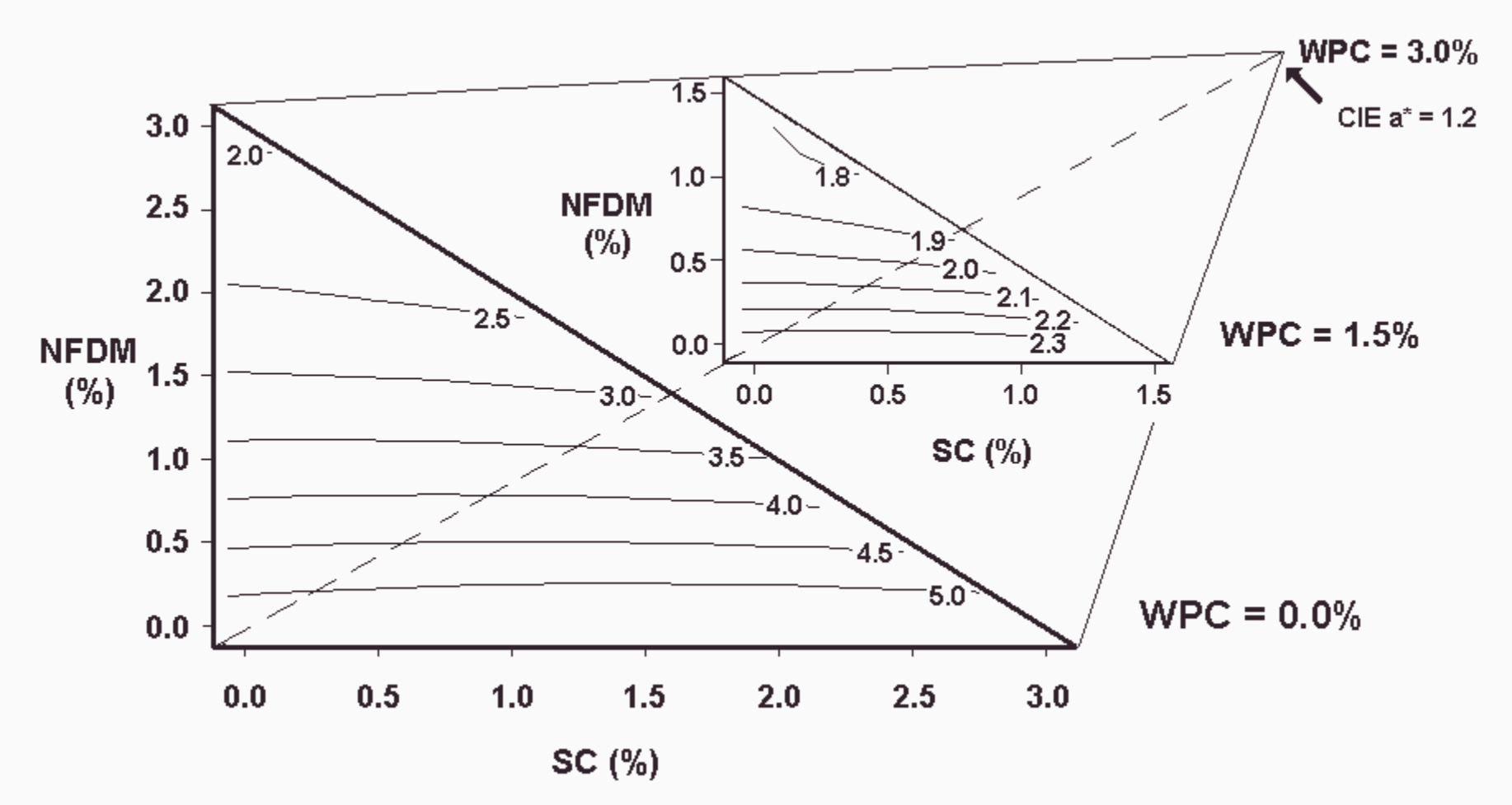

Fig. 5–Contour plots of CIE a* (redness) for samples containing the dairy proteins nonfat dry milk (NFDM), sodium caseinate (SC), and whey protein concentrate (WPC) alone, or in combinations of up to 3.0% added protein with 2.0% nicotinamide added.

10 PPM SODIUM NITRITE

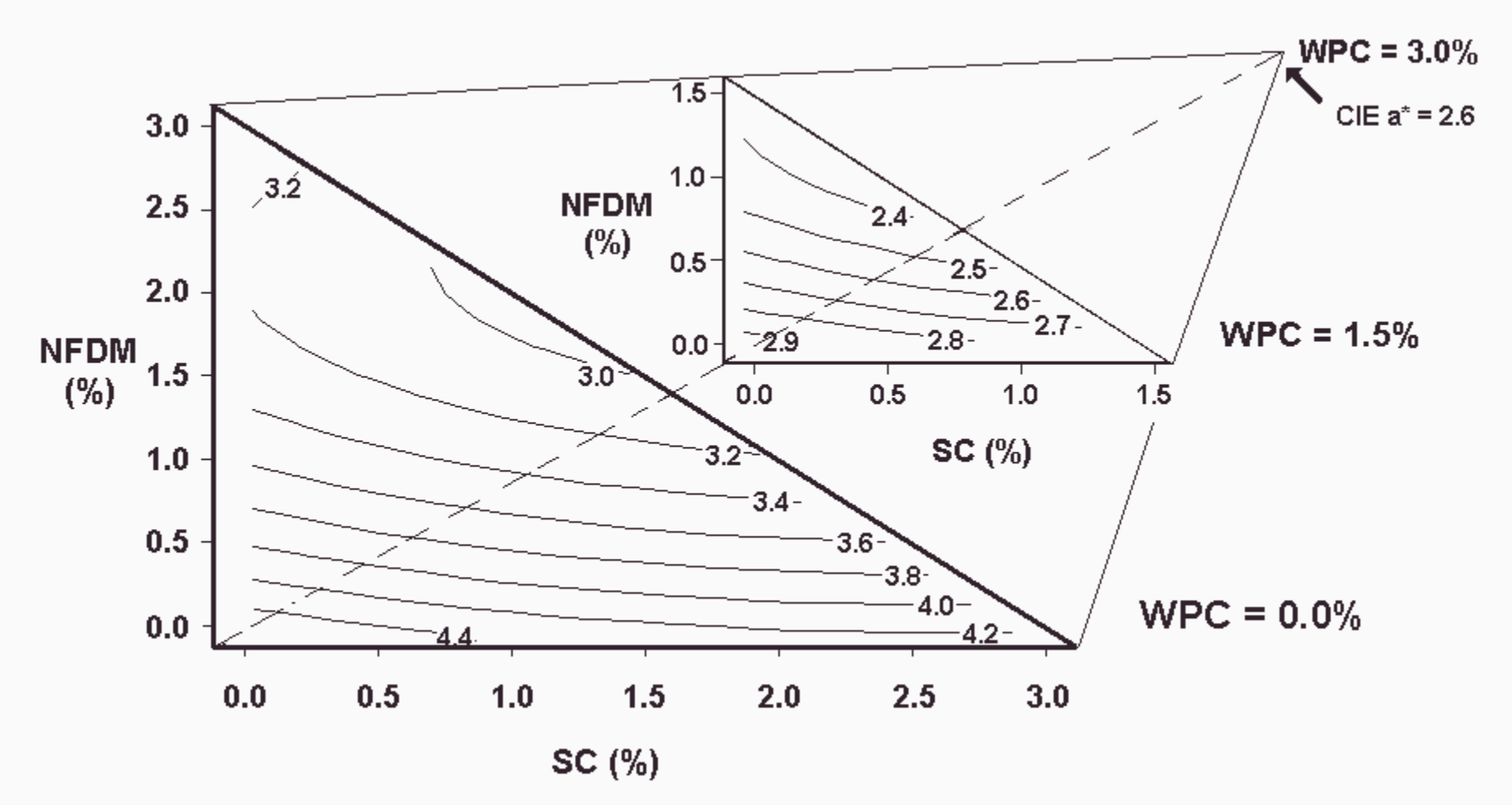

Fig. 6–Contour plots of CIE a* (redness) for samples containing the dairy proteins nonfat dry milk (NFDM), sodium caseinate (SC), and whey protein concentrate (WPC) alone, or in combinations of up to 3.0% added protein with 10 ppm sodium nitrite added.

Chapter 6

Summary and Conclusions

The pink color defect has the potential to be reduced or eliminated by the incorporation of specific dairy proteins. Caution must be made on the exact dairy protein product being used, because some dairy proteins may increase the pink color of the finished poultry product. From the research presented here, and other supporting research, nonfat dry milk and the whey protein concentrate tested in Chapter 5, appear to have relatively strong abilities to reduce pink color. Producers justifying the use of these proteins for their water holding and textural properties could also gain the benefit of reducing the pink color defect.

Further research should focus on the nonfat dry milk and the whey protein concentrates. Determining why only some of the dairy proteins have an effect on pink color could lead to the mechanism(s) involved. Additionally, determining specific differences in the manufacture and detailed composition of these proteins may also aid in developing a product that will completely eliminate the pink color defect.

Chapter 7

Appendix

Tables 1 through 4 are included to complete the color data for the comprehensive dairy protein testing (Chapter 5).

Table 1 – CIE L* values for turkey samples containing dairy proteins in the presence of no pink color generating ligand, nicotinamide (1.0%), nitrite (10 ppm) and nitrate (50 ppm), with and without 200 ppm EDTA present.

Protein (%)	Protein[g]	Ligand			
		No Ligand	Nicotinamide	Nitrite	Nitrate
		NO EDTA			
0	No Protein	78.88^{de}	77.98^{d}	78.36^{ef}	78.67^{c}
3	NFDM	78.96^{d}	78.77^{bc}	78.69^{de}	78.93^{bc}
	SC	77.18^{g}	76.26^{f}	76.86^{h}	77.18^{e}
	WPC	79.93^{a}	79.62^{a}	79.68^{a}	79.93^{a}
1.5	NFDM	79.13^{cd}	78.59^{c}	78.87^{d}	78.95^{bc}
	SC	77.94^{f}	76.90^{e}	77.62^{g}	77.81^{d}
	WPC	79.70^{ab}	79.18^{ab}	79.38^{ab}	79.04^{bc}
1.5 (each)	NFDM + SC	78.52^{e}	77.83^{d}	78.22^{f}	78.99^{bc}
	NFDM + WPC	79.44^{bc}	79.13^{ab}	79.28^{bc}	79.24^{b}
	SC + WPC	79.08^{cd}	78.65^{bc}	79.00^{cd}	79.10^{bc}
0.75 (each)	NFDM + SC + WPC	79.05^{cd}	78.55^{c}	78.82^{d}	79.01^{bc}
	LSD	0.409	0.534	0.367	0.437
		200 ppm EDTA			
0	No Protein	78.76^{d}	78.01^{e}	78.77^{de}	78.83^{cd}
3	NFDM	78.95^{cd}	78.60^{cd}	78.86^{cde}	78.66^{d}
	SC	77.16^{f}	76.24^{g}	77.04^{g}	77.31^{f}
	WPC	79.81^{a}	79.66^{a}	79.76^{a}	79.87^{a}
1.5	NFDM	79.16^{bc}	78.79^{c}	79.05^{bcd}	79.11^{bc}
	SC	77.93^{e}	77.00^{f}	78.02^{f}	77.99^{e}
	WPC	79.81^{a}	79.38^{ab}	79.41^{ab}	79.72^{a}
1.5 (each)	NFDM + SC	78.77^{d}	78.14^{de}	78.64^{e}	78.69^{d}
	NFDM + WPC	79.49^{ab}	79.41^{ab}	79.21^{bc}	79.32^{b}
	SC + WPC	79.34^{b}	78.98^{bc}	79.15^{bcd}	79.09^{bc}
0.75 (each)	NFDM + SC + WPC	79.33^{b}	78.97^{bc}	79.02^{bcde}	79.32^{b}
	LSD	0.349	0.504	0.392	0.388

[a-f] Means within a column and EDTA treatment group (none vs. 200 ppm) with unlike superscript letters are different (P<0.05). Means were pooled over end-point cooking temperature, storage time, and air and light exposure.

[g] Dairy proteins tested include nonfat dry milk (NFDM), sodium caseinate (SC) and whey protein concentrate (WPC).

Table 2 – CIE L* values at two endpoint cooking temperatures (C) and three storage times (d) with and without the presence of 200 ppm EDTA.

			Ligand		
		No Ligand	Nicotinamide	Nitrite	Nitrate
			NO EDTA		
End Point Temperature	74	78.65[b]	78.07[b]	78.45[b]	78.56[b]
	80	79.13[a]	78.56[a]	78.78[a]	79.05[a]
	LSD	0.106	0.138	0.095	0.113
Storage time	1	79.08[a]	78.65[a]	79.03[a]	79.03[a]
	20	78.85[b]	78.14[b]	78.55[b]	78.72[b]
	40	78.74[b]	78.14[b]	78.26[c]	78.66[b]
	LSD	0.155	0.202	0.139	0.166
			200 ppm EDTA		
End Point Temperature	74	78.73[b]	78.28[b]	78.66[b]	78.70[b]
	80	79.18[a]	78.66[a]	78.97[a]	79.11[a]
	LSD	0.090	0.130	0.101	0.100
Storage time	1	79.25[a]	78.90[a]	79.23[a]	79.18[a]
	20	78.81[b]	78.33[b]	78.75[b]	78.79[b]
	40	78.80[b]	78.19[b]	78.46[c]	78.74[b]
	LSD	0.132	0.191	0.149	0.147

[a-c] Means within a column, EDTA treatment group (none vs. 200 ppm), and end point temperature or storage time with unlike superscript letters are different ($P<0.05$). Means for end-point temperature were pooled over air and light exposure, storage time and dairy proteins. Means for storage time were pooled over air and light exposure, end-point temperature, and dairy proteins

Table 3 – CIE b* values for turkey samples containing dairy proteins in the presence of no pink color generating ligand, nicotinamide (1.0%), nitrite (10 ppm) and nitrate (50 ppm), with and without 200 ppm EDTA present.

Protein (%)	Protein[g]	No Ligand	Ligand		
			Nicotinamide	Nitrite	Nitrate
		NO EDTA			
0	No Protein	9.08^{d}	7.60^{c}	7.60^{d}	8.96^{e}
3	NFDM	10.14^{a}	9.81^{a}	8.96^{bc}	10.07^{a}
	SC	9.18^{d}	7.57^{c}	7.53^{d}	9.21^{e}
	WPC	9.98^{ab}	9.78^{a}	9.66^{a}	9.89^{abc}
1.5	NFDM	9.78^{bc}	8.93^{b}	8.64^{c}	9.70^{cd}
	SC	9.17^{d}	7.81^{c}	7.61^{d}	9.19^{e}
	WPC	9.67^{c}	9.02^{b}	9.00^{bc}	9.50^{d}
1.5 (each)	NFDM + SC	9.78^{bc}	8.87^{b}	8.86^{c}	9.79^{bc}
	NFDM + WPC	10.02^{ab}	9.77^{a}	9.45^{ab}	10.06^{ab}
	SC + WPC	9.75^{bc}	9.20^{b}	9.13^{bc}	9.69^{cd}
0.75 (each)	NFDM + SC + WPC	9.69^{c}	9.17^{b}	9.06^{bc}	9.64^{cd}
	LSD	0.279	0.502	0.503	0.278
		200 ppm EDTA			
0	No Protein	10.35^{ab}	9.75^{ab}	10.09^{abc}	10.31^{ab}
3	NFDM	10.29^{abc}	9.93^{a}	9.67^{cd}	10.02^{bcd}
	SC	10.29^{abc}	9.59^{abc}	10.36^{a}	10.40^{a}
	WPC	10.23^{abc}	9.92^{a}	9.88^{bcd}	10.12^{abcd}
1.5	NFDM	10.00^{c}	9.33^{bcd}	9.55^{d}	9.93^{d}
	SC	10.46^{a}	9.74^{ab}	10.26^{ab}	10.36^{a}
	WPC	10.06^{bc}	9.40^{bcd}	9.67^{cd}	9.97^{cd}
1.5 (each)	NFDM + SC	10.10^{bc}	9.05^{d}	9.71^{cd}	9.97^{cd}
	NFDM + WPC	10.29^{abc}	10.03^{a}	9.86^{bcd}	10.23^{abc}
	SC + WPC	10.17^{abc}	9.29^{bcd}	9.81^{cd}	10.01^{cd}
0.75 (each)	NFDM + SC + WPC	10.05^{c}	9.26^{cd}	9.72^{cd}	10.00^{cd}
	LSD	0.299	0.466	0.425	0.297

[a-f] Means within a column and EDTA treatment group (none vs. 200 ppm) with unlike superscript letters are different (P<0.05). Means were pooled over end-point cooking temperature, storage time, and air and light exposure.

[g] Dairy proteins tested include nonfat dry milk (NFDM), sodium caseinate (SC) and whey protein concentrate (WPC).

Table 4 – CIE b* values at two endpoint cooking temperatures (C) and three storage times (d) with and without the presence of 200 ppm EDTA.

			Ligand		
		No Ligand	Nicotinamide	Nitrite	Nitrate
			NO EDTA		
End Point Temperature	74	9.56[b]	8.74[b]	8.74[a]	9.46[b]
	80	9.76[a]	8.99[a]	8.62[a]	9.76[a]
	LSD	0.072	0.130	0.130	0.072
Storage time	1	9.85[a]	9.39[a]	9.34[a]	9.83[a]
	20	9.68[b]	8.78[b]	8.54[b]	9.65[b]
	40	9.45[c]	8.43[c]	8.16[c]	9.35[c]
	LSD	0.106	0.190	0.191	0.106
			200 ppm EDTA		
End Point Temperature	74	10.02[b]	9.50[b]	9.77[b]	9.95[b]
	80	10.39[a]	9.64[a]	9.97[a]	10.30[a]
	LSD	0.077	0.121	0.110	0.077
Storage time	1	10.20[ab]	10.11[a]	10.11[a]	10.15[a]
	20	10.30[a]	9.54[b]	10.00[a]	10.21[a]
	40	10.12[b]	9.07[c]	9.50[b]	10.01[b]
	LSD	0.113	0.177	0.161	0.113

[a-c] Means within a column, EDTA treatment group (none vs. 200 ppm), and end point temperature or storage time with unlike superscript letters are different (P<0.05). Means for end-point temperature were pooled over air and light exposure, storage time and dairy proteins. Means for storage time were pooled over air and light exposure, end-point temperature, and dairy proteins

Vita

Alan John Slesinski was born on September 28, 1972 in Manhattan, Kansas and is the son of Ronald and Mary Jane Slesinski. He received his high school diploma from Franklin Regional High School in Murrysville, Pennsylvania and an Associate of Occupational Science degree in Culinary Arts from Johnson & Wales University. In 1996, Alan received his Bachelor of Science degree in Food Science and Technology from Virginia Tech. After graduation Alan moved to New Jersey where he began employment with Bestfoods North America as a Food Technologist.