MARKETING AND PRODUCTS

Effects of Added Nitrites, Sodium Chloride, and Phosphate on Color, Nitrosoheme Pigment, Total Pigment, and Residual Nitrite in Oven-Roasted Turkey Breast

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ABSTRACT Sixteen combinations of nitrite (0, 1. 10, 50 parts per million (ppm)), sodium chloride (0, 2%), and phosphate (0, .5%) were mixed with turkey breast meat before cooking to determine the main effects and interactions on color (redness, a; yellowness, b; lightness, L) value, nitrosoheme pigment, total pigment, and residual nitrite in oven-roasted turkey breast meat. Addition of 1 ppm nitrite caused a significant color change in oven-roasted turkey breast meat. By increasing nitrite level, a values, nitrosoheme pigment, total pigment, and residual nitrite significantly (P<.01) increased, but b and L values significantly decreased. No differences were found between parameters at the 10 ppm and 50 ppm levels of added nitrite except for that of residual nitrite.

Sodium chloride increased a values (P<.05) and residual nitrite but significantly decreased b and L values. Sodium chloride also decreased nitrosoheme pigment and total pigment in nitrite-treated meat. Phosphate decreased L values but increased (P<.01) residual nitrite in the samples treated with 10 ppm and 50 ppm nitrite.

A salt × phosphate interaction decreased L values at all nitrite levels except that of 10 ppm added nitrite, but significantly (P<.01) increased a values and decreased b values in the samples with 50 ppm added nitrite. Total pigment was significantly (P<.05) increased by a salt × phosphate interaction in the 0 and 1-ppm nitrite samples, but residual nitrite was increased only in the 10 and 50-ppm nitrite samples.

(Key words: nitrite, color, nitrosoheme, turkey, breast)

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INTRODUCTION

The pink color in oven-roasted turkey breast meat is of great concern to the turkey meat industry as well as to consumers. Usually the doneness of cooked meat is judged on the basis of meat color. In oven-roasted turkey breast meat neither nitrite nor nitrate, which are used for Clostridium botulinum control and pink color development, are added. Therefore, the pink color after oven roasting could be suspected of resulting from undercooked or contaminated meat. Although this pinkness is a cosmetic problem, it can greatly affect the purchasing behavior of consumers.

Various studies have attempted to determine the cause of this color problem. Pool (1956) reported that carbon monoxide (CO) and nitric oxide (NO) generated by flames can cause a pink color in the uncovered roasting of birds. The effects of end-point cooking temperature and color regeneration were studied by Helmke and Froning (1971). They reported that redness of cooked meat was significantly decreased at higher end-point temperatures, and a regenerated pink pigment appeared to develop within a 2-h storage period. Cornforth et al. (1986) insisted that the meat redox potential is the most important factor in the pink color of cooked turkey rolls. Janky and Froning (1973) reported that a lowering of the pH resulted in a decrease in stability of the myoglobin (Mb) derivatives, and metmyoglobin was more stable than either Mb or oxymyoglobin (Mbo2). Addition of Kena significantly increased the stability of all three myoglobin derivatives by increasing pH. Aluminum foil-cooked chicken white meat from birds fed 600 parts per million (ppm) nitrate (NO3) and open-cooked dark meat from turkeys fed 100 ppm nitrite (NO2) were found to have significantly higher a values than those of the control (Froning et al., 1969). The level of NO3 nitrogen in drinking water had no significant effect (P<.01) on turkey meat color, but NO3 nitrogen in the chill water and cooking significantly (P<.01) increased redness (a value)
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(Mugler et al., 1970). Significant color development was observed when carcasses were held for more than 3 days at 4°C in water or ice containing 3 ppm NO₂ or 50 ppm NO₃ (Nash et al., 1985).

Froning et al. (1968) reported that the redness ($a_l$ values) of raw turkey meat significantly increased with age, and cooked meat from male birds exhibited significantly lower L values than those of the female birds. Ngoka et al. (1982) reported that free struggle could cause some pink color, and pre-slaughter excitement caused a darker color and redness (lower L and higher $a_l$ values) in the breast meat. Meat color from heat-stressed birds had higher L values compared with those of control or cold pre-slaughter treatments (Babji et al., 1982). Objectives of this experiment were 1) to determine the level of added NO₂ that can cause a pink color problem, and 2) to investigate how the added NO₂, sodium chloride, and phosphate affect the color of oven-roasted turkey breast meat.

MATERIALS AND METHODS

Preparation of Meat Samples. A $4 \times 2 \times 2$ factorial, completely randomized block design was used. Four levels of NO₂ (0, 1, 10, 50 ppm), two levels of NaCl (0, 2%), and two levels of sodium tripolyphosphate (0, .5%) were used for a total of 16 combinations of 24-h post-mortem turkey breast meat samples (ca .5 kg each). Each combination of additives was dissolved in distilled water and the resulting solution (15%; meat weight basis) was injected with a syringe into the meat samples and mixed with a KitchenAid mixer (model K5-A) for 2 min. Meat preparations were wrapped with aluminum foil and stored for 2 h in a 4°C cooler for equilibration of additives in the meat. Prepared samples were cooked in an electric oven starting at 65°C followed by 5°C temperature increases every 1 h until a final meat temperature of 72°C was reached. The experiment was repeated four times.

Color Measurement. Color evaluation of oven-roasted meat was measured at room temperature (21°C) by using a Hunter lab color difference meter (Hunter Associates Laboratories, Inc., Reston, VA) with a D25-9 reference. The colorimeter was standardized with a white ceramic tile having values: L, 92.14; a, -1.20; and b, .10. Slices ca 1 cm thick were placed on a clean glass; color readings were taken, of redness (a), yellowness (b), and lightness (L). Two slices per sample were made, and two readings per slice were taken with ca 90° rotation between readings.

Nitrosoheme and Total Pigment Absorption Spectrophotometry. Amounts of nitrosoheme pigment and total pigment in oven-roasted turkey breast meat were measured using Hornsey’s method (1956). Nitrosoheme pigment and total pigment were extracted from the oven-roasted turkey breast meat with 80% acetonitrile or acetone-HCl solution as described by Hornsey (1956) and filtered through a .45-μm Prep-Disc membrane filter (Bio-Rad, Richmond, CA). Absorbances were measured at 540 nm for nitrosoheme pigment and 630 nm for total pigment, with a spectrophotometer (Model 34, Beckman Instruments, Inc., Fullerton, CA).

To obtain the relationship between nitrosoheme pigment and total pigment, nitrosoheme pigment and total pigment were extracted from commercial turkey frankfurters using the same method as for the oven-roasted turkey breast meat. Absorption spectra of the nitrosoheme and total pigment were recorded from 700 to 450 nm. A few drops of HCl were added to the nitrosoheme pigment, and the absorption spectrum of the resulting solution was also recorded.

Residual Nitrite Measurement. Residual nitrite was measured using the Association of Official Analytical Chemists (1980) method, with some modifications. Ten grams of finely comminuted meat were mixed with 90 mL distilled water and heated for 2 h in an 80°C water bath. After cooling with ice water to room temperature, the volume of the solution was adjusted to 100 mL. Fifty milliliters of the resulting solution were decanted to a 250-mL beaker and 2 mL of Carrez II (30% ZnSO₄·7H₂O), and 2 mL of Carrez I (15% K₄Fe(CN)₆·3H₂O) solutions were added to precipitate proteins. The volume was then adjusted to 150 mL and mixed thoroughly.

After protein precipitation, the clear supernatant solution was drawn off with a 50-mL syringe and filtered through a .2 or .45 μm membrane filter. Ten to 45 mL of solution were collected in a 100-mL flask and then 2.5 mL of sulfanilamide and . 2.5 mL of naphthylethylenediamine (NED) reagent were added. The final volume was adjusted to 50 mL. After 15 min of color development, the absorbance at 540 nm was measured with a spectrophotometer (Beckman, model 34).

Statistical Analysis. Analysis of variance was performed with the use of the statistical analysis system (SAS, 1986) to determine differences
due to the main effects of NO₂, salt, and phosphate and their interactions. The values for main effects in the tables were expressed as the increase or decrease in each parameter caused by the added ingredient (e.g., for a salt main effect, an a value of .7 (average of 3.93 in Table 1, 0 ppm nitrite) means the actual a value with added salt was 4.28, and 3.58 without salt. For the interaction effect, the actual a value of (0% salt × 0% phosphate) + (2% salt × .5% phosphate)/2 was 3.96, and that of (0% salt × .5% phosphate) + (2% salt × 0% phosphate)/2 was 3.90. Duncan’s multiple range test was used to determine significant differences in mean values (Steel and Torrie, 1960). Linear regression equations were calculated using the statistical analysis system (SAS, 1986) to determine relationships among a values, nitrosoheme pigment, and total pigment.

### Results and Discussion

**Effects of NO₂.** Table 1 shows the effects of added NO₂, salt, and phosphate on color values, nitrosoheme pigment, total pigment, and residual NO₂ in oven-roasted turkey breast meat. Increased addition of NO₂ (NaNO₂) significantly (P.<.01) increased a values, nitrosoheme pigment, total pigment, and residual nitrite, but significantly decreased b and L values (P.<.01). No differences were found between parameters at levels of 10 ppm and 50 ppm added NO₂ except for residual NO₂. At the level of 10 ppm added NO₂ the maximum amount of nitrosoheme pigment was formed, and still more than 2 ppm residual NO₂ were observed. Addition of more than 10 ppm NO₂ did not increase redness, nitrosoheme pigment, or total pigment in turkey breast meat. Addition of 1 ppm NO₂

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\( ^a-c \) Means within parameters with no common superscripts are significantly different (P.<.05).

\( ^1 \) Changes caused by adding the respective ingredient compared with values for all samples without the ingredient.

\( ^* \) Significant at P.<.05.

\( ^** \) Significant at P.<.01.
significantly increased (P<.01) the a values in turkey breast meat.

The increase in total pigment with increasing addition of NO₂ was correlated with nitrosoheme pigment levels. This relationship can be expressed by the linear regression equation y = .7x + 23.1, where y is total pigment and x is nitrosoheme pigment.

Total pigment increase due to nitrosoheme pigment in NO₂-treated meat can be explained by the two-step reaction of heme pigments. The first step is the formation of nitrosylmyoglobin or hemoglobin (Hb). The added NO₂ can be converted to NO by cytochrome c (Walters and Taylor, 1965), nicotinamide adenine dinucleotide (NADH), cysteine, and ascorbate (Fox and Ackerman, 1968). In his review paper, Giddings (1977) stated that both ferrous and ferric Mb will combine with NO to yield the same pigment. The latter is believed to autoreduce with time via internal electronic rearrangement. Further, ferrous Mb can reduce NO₂ to generate NO, which will combine with either oxidation state of the pigment. Endogenous or added reductants such as ascorbate, sulfhydryl compounds, and NADH-flavins accelerate the process by either reducing ferrimyoglobin, which can then reduce NO₂, or reducing NO₂ to NO, or both, in addition to perhaps accelerating the reduction of ferric Hb-NO. The reaction rate of NO with Hb and Mb is 100-fold greater than that of CO, which, in turn, has a much greater affinity to Hb or Mb than NO₂. Therefore, the amount of nitrosylmyoglobin or Hb is mostly decided by the amount of Mb or Hb and NO in meat.

The second step in reaction of heme pigments is the conversion of nitrosoheme pigment to total pigment. At very low pH (pH = 1), adjusted with HCl, nitrosoheme pigment changes from a pink-red color to a brownish acid hematin (chlorohememin) color. The fifth and sixth ligands of nitrosoheme pigment are occupied by NO (Tarladgis, 1962; Lee et al., 1976) and only the fifth ligand of acid hematin (chlorohememin) is occupied by a chloride ion. It was observed that the absorption spectra of nitrosoheme pigment + HCl was essentially the same as that of total pigment extracted with acetone-HCl solution (Figure 1). When the amounts of the two pigments were calculated from their absorbance at 540 nm, for nitrosoheme pigment, and 640 nm, for total pigment, by using Hornsey's (1956) extinction coefficient, their values were nearly the same (111.07 ppm for nitrosoheme pigment and 112.88 ppm for total pigment). Figure 1 also shows that the amount of total pigment extracted directly from turkey franks is greater than that converted from nitrosoheme pigment. In uncured meat no NO is available, but it can be assumed that there are still some materials that can react with heme of Hb or Mb. Soluble proteins, amino acids, NADH, and amides could fall into that category. They could be a ligand of Hb or Mb if the heme of Mb or Hb is exposed to them after heat denaturation (Livingstone and Brown, 1981). Akoyunoglu et al. (1963) showed that many amino acids and their derivatives could react with heme or hematin and form ferrihemochromes and ferrohemochromes. Ledward (1974) insisted that the heme proteins in cooked meat are mainly a di-imidazole complex, the imidazole residues being supplied by the histidine groups of the bound protein. These compounds can exert a very strong effect on color, depending on their reaction with heme pigments. Some of them are insoluble in acetone-HCl solution. One example of this is the Mb-NADH complex. Results from our laboratory have shown that after heat denaturation, the Mb-NADH complex gives a brownish-green color and is much less soluble than Mb in acetone-HCl solution.

The heme of cytochrome c was not extractable with acetone-HCl solution because of the differences between Hb/Mb and cytochrome c in their heme environment (Takano et al., 1973). Both the fifth and sixth ligands of heme in cytochrome c are covalently bound to amino acid side chains (fifth ligand with histidine, sixth ligand with methionine). Only the fifth ligand of Mb and Hb is covalently bound to histidine. Therefore, it can be concluded that total pigment is only the sum of heme converted from nitrosoheme pigment, and Mb and Hb not reacted with NO or other materials such as proteins, amino acids, amide, and NADH.

When levels greater than 10 ppm NO₂ were added, about 30% of the added NO₂ was detected as residual NO₂ in turkey breast meat in the present study. When 156 ppm of NO₂ were added to pork, about 37% of the residual NO₂ was observed after 2 days of storage at 71 °C (Sebranek et al., 1973). The amount of NO₂ (NaNO₂) converted to NO and bound to nitrosoheme pigment, calculated from samples from the 10 ppm NO₂ treatment, was about 4 ppm. Sebranek et al. (1973) reported that about 16 ppm of NO₂ (NaNO₂) were converted to NO and bound to heme pigments in pork. This dif-
ference might be caused by the variation in the amount of heme pigments in pork and turkey breast meat.

The a value increased with increasing nitrosoheme pigment and total pigment levels (P<.01). For the a value and nitrosoheme pigment relationship, the linear regression equation was $y = 0.57x - 11.98$, where $y$ is the a value and $x$ is nitrosoheme pigment. The linear regression equation for a value and total pigment was $y = 0.79x - 3.01$, where $y$ is a value and $x$ is total pigment.

**Effects of Salt.** Added salt significantly (P<.05) increased a values at all levels of NO₂ (Table 1). With nitrite plus salt, both amounts of nitrosoheme pigment and total pigment were significantly decreased (P<.01 and P<.05). Residual NO₂ was significantly (P<.01) increased by addition of salt in the 10 ppm and 50 ppm NO₂ treatments.

The primary explanation for the increase in residual NO₂ from added salt might be related to the inhibition of enzyme activities in electron transport chains and metmyoglobin reductase (Stewart et al., 1965). Less conversion of NO₂ to NO may have resulted in less nitrosylmyoglobin or Hb formation. Therefore, less nitrosoheme pigment formation could be the primary cause of low total pigment. An experiment conducted in the authors' laboratory comparing several inhibitors (rotenone, potassium cyanide, and antimycin) showed that the electron transport chain could contribute up to 40% of the total NO₂ converted, and 10% added salt decreased nitrosoheme pigment by 60% and increased residual NO₂ by 15%. Walters and Taylor (1965) showed that the cytochrome oxidase in skeletal muscle mitochondria is responsible for reduction of NO₂ to NO in anaerobic conditions.

The secondary but very important explanation for redness resulting from added salt in meat with and without added NO₂ could be the reaction of solubilized myofibrillar proteins and

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**FIGURE 1.** Absorption spectra of nitrosoheme pigment, nitrosoheme + HCl, and total pigment from turkey franks.
heme pigments. Added sodium chloride can solubilize the myofibrillar proteins. Perhaps a larger amount of protein became available for reaction with heme; this can create a greater chance for heme pigment to react with proteins or other materials if the heme or Hb or Mb is exposed by heat denaturation (Livingstone and Brown, 1981). The increase in a value with added salt supports the hypothesis that there are some other materials that can affect a values but which are not extractable with acetone and acetone-HCl solution.

The third explanation of the effects of added salt on meat color could be the effect of cytochrome c. Cytochrome c was not extractable with acetone and acetone-HCl solution. The proportion of cytochrome c in some avian breast meat pigment is unusually high compared with that found in other types of meat (Schollemeyer and Klingenberg, 1962). The extinction coefficient of cytochrome c at 550 nm, which gives a pink red color, is about three-fold higher than that of Mb after reduction (Van Gelder and Slater, 1962; Hardman et al., 1966). Although the Mb is 50-fold higher than cytochrome c in breast meat, the contribution of cytochrome c to pinkness of breast meat could be higher than that of Mb in cooked meat. Ahn et al. (1986) reported that 2.5% NaCl greatly increased the heat stability of cytochrome c but decreased that of Mb in 1-M phosphate buffer solution (pH 6.3). Sodium chloride significantly decreased b and L values. Both b and L values were inversely related to a values.

Effects of Phosphate. The amount of residual NO₂ was significantly (P<.01) increased by phosphate addition in the 10 ppm and 50 ppm NO₂ treatments. Although significant increases and decreases were shown with 0 and 1 ppm NO₂ addition (Table 1), they have no practical meaning.

The .5% added phosphate could increase pH by 2 units in an unbuffered solution and .4 unit in turkey breast meat. As NO formation from HNO₂ is favorable in acid conditions, an increase in pH (decrease in H⁺ concentration) would result in unfavorable conditions for the following half reaction: HNO₂ + H⁺ + e⁻ → NO (g) + H₂O; E° = -1.0 V.

Lee et al. (1976) reported that white muscle, which has a lower pH than that of red muscle, contained less residual NO₂ than the amount found in red muscle. Prusa and Kregel (1985) reported that an increase in pH could cause an increase in residual NO₂. Nitrosoheme pigment was significantly decreased in breast meat from the 1-ppm NO₂ treatment. The L value was also significantly decreased by adding phosphate. Froning (1965) reported that uncooked polyphosphate-treated meat had a bluish-white appearance.

Salt × Phosphate Interactions. The a value of turkey breast meat was significantly (P<.01) increased but the b value was decreased by a salt × phosphate interaction in the 50 ppm NO₂ treatment (Table 1). The highest a value was obtained with the 2% salt × .5% phosphate combination, and it was significantly higher than that of the other three. The highest b value was obtained with the 0% salt × .5% phosphate combination, and the lowest was .5% phosphate × 2% salt; the b values were significantly different. The L values were significantly decreased by the salt × phosphate interaction at all levels of NO₂ except for 10 ppm NO₂. The L values were the lowest for the 2% salt × .5% phosphate combination.

A total pigment increase was produced by the salt × phosphate interaction in the 0 ppm and 1-ppm nitrite treatments. When no NO₂ was added, the highest total pigment was obtained with the 2% salt × .5% phosphate combination. With 1 ppm NO₂, the 0% salt × 0% phosphate combination produced the highest total pigment.

Salt × phosphate interactions significantly increased residual NO₂ when 10 and 50 ppm of NO₂ were added, but decreased residual NO₂ when 0 and 1 ppm NO₂ were added. In the 10 ppm and 50 ppm NO₂ treatments, the 2% salt × .5% phosphate combination produced the highest residual NO₂; this combination allowed significantly (P<.01) higher residual NO₂ than any other salt × phosphate combination.

Overall, NO₂ had the most powerful effect on the parameters measured in this study. Even 1 ppm added NO₂ produced significant differences in all color parameters. Sodium chloride showed a very strong effect on color. As NO₂ is not added in oven-roasted turkey breast meat, NaCl may be the most important added ingredient affecting oven-roasted turkey breast meat color. Phosphate also can affect color, but its effect was much smaller than that of NaCl in the present research.

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