

## Research Article

## Influence of Nitrate-nitrite Contamination on Pink Color Defect in Ginger Marinated Steamed Chicken Drumsticks

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### Abstract

This study aimed at investigating influence of nitrate-nitrite contamination in ginger on pink color defect in marinated steamed chicken drumsticks. Three marinade formulations representing different degrees of nitrate-nitrite contamination were explored (i.e. no ginger (0 ppm) – A, ginger paste #1 containing 30 ppm nitrate – B, and ginger paste #2 containing 30 ppm nitrate + 8 ppm nitrite – C, respectively). Total myoglobin content was measured in chicken drumsticks before marination. Nitric oxide myoglobin (NOMB) was determined in the samples both before and after marination. After cooking, occurrences of pinking,  $a^*$  value and nitrosyl hemochrome (NHC) content were evaluated. It was found that pink color defect was observed in treatment C. These results indicated that the presence of nitrite in ginger (treatment C) played a significant role in pinking appearance of steamed chicken drumstick. The maximum nitrite contamination in chicken drumstick was also investigated using sodium nitrite solution and it was found that it should not be more than 0.8 ppm. Thus, it is crucial to control the amount of nitrite contamination in ginger paste to avoid pink color defect formation.

**Keywords:** Chicken drumstick, Ginger, Pink color, Nitrate-nitrite contamination, Nitrosyl hemochrome

### 1 Introduction

Pink color defect is a complex quality problem in fully cooked meat products which often influences consumers' confidence on product safety. Consumers often associate color and appearance of product to its quality. They expect raw poultry meat to be pink, while the fully cooked one should become white or brown. In fully cooked chicken meat, the presence of pink

color is a major concern. Although it was not a food safety issue, many consumers may think that it was undercooked and unsafe for consumption [1]. This problem presents a food quality issue as well as an economic concern to the food industries for almost 40 years [2]. Since Thailand is one of the major producers of ready-to-eat chicken products exported to several countries worldwide [3], the problem of pink color defect could greatly impact the economic loss of the

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Thai poultry processors. Due to the sporadic nature of pink color defect occurrence, there is no adequate information that could lead to the root-cause of this problem.

There are several factors that can contribute to pink color problem such as bird age, preslaughter stress, feed formulation, or consumption of moldy feed materials [4]–[6]. Mishandling of live birds in farm, during transportation or during slaughtering may cause bruising and hemorrhaging which turn into discoloration defect in the cooked meat [7]. Post-slaughter pinking may cause by several factors such as postmortem aging, nitrate or nitrite in water or ice used for processing, certain ingredients, contamination of nitrate reducing bacteria or cooking methods [8]–[10].

Color of meat comes from pigments presented in muscle, namely myoglobin, hemoglobin and cytochrome c. Pinking of fully cooked meats often associates with pigments derived from denatured globin hemochrome. During cooking, globin denatures and nitrosyl hemochrome complex (NHC) is formed which has a stable pink color. The minimum concentration of sodium nitrite necessary to form detectable pink color were 14 ppm for beef, 4 ppm for pork, 2 ppm for turkey, and 1 ppm for chicken [11]. It was noted that nitrates must be reduced to nitrite in order to form nitric oxide myoglobin (NOMB), which will denature and turn into NHC when heat is applied. There are several nitrate reduction mechanisms, such as bacterial reduction or the reduction capability of the raw meat [10]. Although many researches have been trying to identify the mechanism of pink color development, only few studies were conducted in real food system.

To give flavor to the cooked chicken, several ingredients are often added to raw meat and marinated prior to cooking. Asian marinade for chicken often includes soy sauce, sesame oil, garlic and ginger. Ginger (*Zingiber officinale* Roscoe) is a flowering plant whose root is generally used as a spice and a folk medicine. Since the root of ginger is used for cooking, there is a high risk of nitrate-nitrite contamination from soil and fertilizer used during plantation. In addition, ginger can produce nitrite by oxidation of endogenous nitrogen oxides, and nitrate can be reduced by nitrite reductase to produce nitrite [12]. Nitrite content in ginger varied from 0.0732–0.4651 mg/kg dry weight [13]. Although nitrite itself is relatively non-toxic, it can be a precursor material of nitrosyl hemochrome.

Thus, this study aimed at investigating influence of nitrate-nitrite contamination in ginger on pink color defect in ginger marinated steamed chicken drumsticks.

## 2 Material and Methods

### 2.1 Material preparation

Fresh chicken drumsticks were obtained from a commercial broiler processing plant. Two hundred grams of chicken drumstick were individually packed in polyethylene bags and stored at 4°C for approximately 48 h prior to the experiments. Before marination, the drumsticks were cut into small pieces with an approximate weight of 15±2 g/piece and dimensions of 4 cm width × 4.5 cm length × 3 cm thickness.

Ginger pastes were purchased from two local suppliers and stored at 4°C until use.

### 2.2 Evaluation of the effect of nitrate-nitrite contamination in ginger on pink color defect

#### 2.2.1 Marination of chicken drumsticks

Three marinade formulations were used in this study: formula A with no ginger (0 ppm), formula B with ginger paste from supplier #1 (containing 30 ppm nitrate), and formula C with ginger paste from supplier #2 (containing 30 ppm nitrate + 8 ppm nitrite). The marinades contain 22% of ginger paste and 78% of soy sauce-based seasoning sauce, except formula A. After 48 h storage, the meat samples were mixed with each marinade (20% of meat weight) in a polyethylene (PE) bag for 35 min and stirred every 10 min interval to simulate a tumbling process. After that, still marination was performed by placing the PE bags in a refrigerator (4–5°C) for 6 h.

#### 2.2.2 Cooking of chicken drumsticks

The chicken drumsticks were cooked by steaming. Prior to cook, a steamer was pre-heat until the water was boiled before placing the samples on a perforated tray. The marinated samples were steamed until its core temperature, which was measured at the geometric center of the sample, reached 80°C. After cooking, the sample was cooled at room temperature for approximately 30 min before further analysis.

### 2.3 Evaluation of the effect of nitrite content on pink color development in chicken drumsticks

Sodium nitrite solutions were prepared at 0, 4, 8, and 12 ppm and used as marinades. The meats were marinated with sodium nitrite solution at 20% using (meat weight basis) in polyethylene bag for 35 min and stirred every 10 min interval. Then, the samples were steamed within 1 h after marination. The temperature was controlled at 4–5°C throughout the marination process.

### 2.4 Determination of total myoglobin content

Total myoglobin was determined according to Warriss with some modification [14]. Four grams of raw minced meat was placed into a 50 mL polypropylene centrifuge tube, and 20 mL of cold potassium phosphate buffer (pH 6.8, 40 mM, 4°C) was added. The mixture was homogenized for 5 s at 10,000 rpm for 5 times with a high-performance homogenizer (ULTRA-TURRAX®, T25 digital, IKA, Werke Staufen, Germany). The homogenized sample was centrifuged at 7,000 g for 30 min at 4°C (Himac CR22N, Hitachi Co. Ltd., Tokyo, Japan). The supernatant was filtered with 0.45 µm nylon membrane syringe filter (Bonna-Agela Technologies Inc., Delaware, USA). After that, 10 mL of the supernatant was drawn for total pigment measurement using cyanmetmyoglobin (cyanmetMb) method [13].

A few micrograms of potassium ferricyanide and sodium cyanide were added to the supernatant to convert the pigments to the cyanmetMb form. Then, the sample was centrifuged at 24,900 g for 1 h at 4°C. The supernatant was subjected to absorbance measurement at 540 nm using the UV/VIS spectrophotometer where 40 mM of phosphate buffer (pH 6.8) was used as a blank. The extinction coefficient at the absorption maximum (at 540 nm) of the cyanmetMb was 11.3 mm<sup>-1</sup> cm<sup>-1</sup>. Therefore, the total myoglobin concentration in meat extracts was calculated from the absorbance at 540 nm ( $A_{540}$ ) as follows [Equation (1)]:

$$\begin{aligned} & \text{Total myoglobin (mmol/g)} \\ &= \frac{A_{540}}{11.3 \text{ (L/mmol)}} \times \frac{\text{extraction volume (L)}}{\text{sample (g)}} \quad (1) \end{aligned}$$

### 2.5 Determination of nitric oxide myoglobin content

Nitric oxide myoglobin (NOMb) content was measured according to Honsey with some modification [15]. Four grams of the raw minced meat were mixed thoroughly with 22 mL aqueous acetone using a glass stirring rod for 1 min. Then, a beaker was sealed with an aluminum foil to reduce evaporation. The mixture was kept at room temperature under subdued light for 10 min, and then the supernatant was filtrated through Whatman no. 42 filter paper (9 cm in diameter). The supernatant was subjected to absorption measurement at 540 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu corp., Kyoto, Japan) within 1 h. The concentration of NOMb was calculated as follows [Equation (2)]:

$$\begin{aligned} & \text{NOMb (mmol/g)} \\ &= \frac{A_{540}}{11.3 \text{ (L/mmol)}} \times \frac{\text{extraction volume (L)}}{\text{sample (g)}} \quad (2) \end{aligned}$$

### 2.6 Determination of nitrosyl hemochrome content

Like NOMb content, nitrosyl hemochrome (NHC) content was evaluated by the modified method of [15]. Four grams of the cooked sample were minced and mixed with 9 mL of aqueous acetone using a glass stirring rod for 1 min. Subsequently, tube cap was sealed to reduce evaporation. The mixture was kept at room temperature under subdued light for 10 min, and then the supernatant was filtrated through Whatman no. 42 filter paper (9 cm in diameter). The supernatant was directly subjected to measurement of the absorption of nitrosyl hemochrome at 540 nm by using a UV-VIS spectrophotometer (UV-2600, Shimadzu corp., Kyoto, Japan) within 1 h. The concentration of nitrosyl hemochrome was calculated as follows [Equation (3)]:

$$\begin{aligned} & \text{NHC (mmol/g)} \\ &= \frac{A_{540}}{11.3 \text{ (L/mmol)}} \times \frac{\text{extraction volume (L)}}{\text{sample (g)}} \quad (3) \end{aligned}$$

### 2.7 Determination of pink color defect

After the raw and marinated chicken drumsticks were steamed, 6 pieces of the steamed samples were cut in



**Figure 1:** Meat sample prepared for visual inspection and color measurement.

half lengthwise (Figure 1).

Within 30 min after cutting, visual inspection was performed on the inside surfaces to determine pinking appearance in cooked sample. It was noted that the pink color of nitrosyl hemochrome is light and oxygen sensitive [1]. The pink appearance could be faded after long ambient light and air exposure. The number of samples with noticeable pink appearance were counted and percentage of pink color defect was calculated by amount of pink sample divided by total steamed sample multiply by 100 as shown in following Equation (4),

$$\% \text{Pink Color Defect} = \frac{\text{Pink Sample}}{\text{Total Steamed Sample}} \times 100 \quad (4)$$

## 2.8 Color measurement

After visual inspection, color of each sample was measured using a spectrophotometer (ColorQuest XE, HunterLab, Virginia, USA) in a reflective mode and reported as  $L^*$ ,  $a^*$  and  $b^*$  values. The aperture size of 9.5 mm was selected. Color measurement was performed 3 times on each piece of sample.

## 2.9 Determination of nitrate content

Nitrate content in ginger paste was determined using the method described in Cataldo *et al.* with some modification [16]. Mix 0.5 g of ginger paste with 6 mL of deionized (DI) water and heat them in a water bath

at 80°C for 30 min. Then, the mixture was cooled and filtered through Whatman no. 42 filter paper (repeated twice). Then, 0.1 mL of the solution was mixed with 0.4 mL of 5% (w/v) salicylic acid (in pure  $\text{H}_2\text{SO}_4$ ) and kept for 10 min. After that, 9.5 mL of 8% NaOH was added to the solution and wait for 20 min. Absorbance of the solution was measured at 410 nm using a UV-VIS spectrophotometer (UV-2600, Shimadzu corp., Kyoto, Japan).

## 2.10 Determination of nitrite content

Nitrite content was analyzed following [17]. Ten grams of ginger paste was mixed with 5 mL of 50g/L saturated borax solution. Later the solution was mixed with hot water (70°C). The mixture was heated in a water bath for 30 min and cooled afterward. Then, 10.6 g of potassium ferrocyanide was added and followed by 21.9 g of zinc acetate solution to precipitate protein. Finally, 10 mL of the solution was pipetted to mix with 50 mL of distilled water, 10 mL of sulphanimide solution, 6 mL of HCl solution and 2 mL of N-1-naphthylethylene diamine dihydrochloride. Absorbance of solution was measured at 538 nm.

## 2.11 Statistical analysis

In this study, one factor at a time was used for experimental design and two replications were conducted per each study. For all statistical analysis, the quality of data was checked to make sure that they were normally distributed with equal variance. ANOVA and Tukey's multiple range test at  $\alpha = 0.05$  were used to determine effect of nitrate-nitrite contamination and differences among different levels of treatment. All computations were performed by using Minitab for Windows version 18 software (Minitab Inc., Pennsylvania, USA).

## 3 Results and Discussion

### 3.1 Effect of nitrate-nitrite contamination in ginger on pink color defect of steamed chicken drumstick

Three formulations of marinade were used (i.e. no ginger (0 ppm) – A, ginger paste #1 containing 30 ppm nitrate – B, and ginger paste #2 containing 30 ppm nitrate + 8 ppm nitrate – C) to determine effect of nitrate-nitrite contamination in ginger on pink color

defect. Concentration of pigments namely myoglobin, NOMb and NHC found in chicken drumsticks marinated with different formulations was reported in Table 1. It was found that the total myoglobin contents in the raw marinated chicken drumstick obtained from all treatments were not significantly different ( $P > 0.05$ ). However, comparing to other treatments, treatment C resulted in significantly higher NOMb content in the raw meat samples ( $P < 0.05$ ). Similarly, the NHC content in the cooked meat samples obtained from treatment C was also significantly higher than those of treatment A and B ( $P < 0.05$ ). Although, treatment B contained 30 ppm of nitrate, there was no significant difference ( $P \geq 0.05$ ) between the NOMb and NHC contents obtained from this treatment and the NOMb and NHC contents obtained from treatment A, which had no ginger in the marinade.

**Table 1:** Contents of pigments in chicken drumsticks from different treatments

Treatment	Total Myoglobin* <sup>ns</sup> ( $\times 10^5$ mmol/g)	NOMb* ( $\times 10^5$ mmol/g)	NHC** ( $\times 10^5$ mmol/g)
A	11.92 $\pm$ 3.36	0.59 <sup>a</sup> $\pm$ 0.18	0.41 <sup>a</sup> $\pm$ 0.11
B	10.89 $\pm$ 3.10	0.68 <sup>a</sup> $\pm$ 0.18	0.54 <sup>a</sup> $\pm$ 0.11
C	11.07 $\pm$ 3.32	1.01 <sup>b</sup> $\pm$ 0.05	1.45 <sup>b</sup> $\pm$ 0.06

Means with different superscripts in the same column differ significantly ( $P < 0.05$ ).

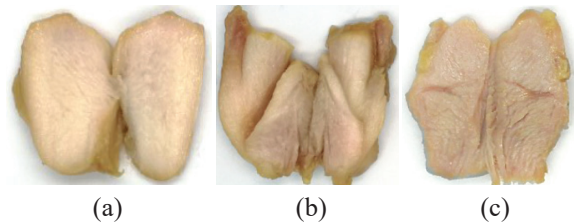
ns – means are not significantly different.

\*Total myoglobin and NOMb were determined in raw chicken meat.

\*\*NHC was determined in cooked chicken meat.

One representative image was selected out of the six cross-section images of steamed chicken drumstick obtained from each treatment and shown in Figure 2. It was found that treatment C resulted in a noticeable pink color defect while treatment A and B resulted in slightly pink color appearance. Although the marinades used in treatment B and C contained the same amount of nitrate (30 ppm), the appearances of steamed chicken drumstick obtained from these treatments were different which might due to different nitrite contents in both treatments.

Color measurement results and percentage of pink color defect occurrence were shown in Table 2. The  $L^*$  value was insensitive to the treatment while the  $a^*$  and  $b^*$  values were significantly affected by the marinade formulations. Similar to the pigment content



**Figure 2:** Appearances of steamed chicken drumstick from treatment (a) A, (b) B and (c) C.

results, treatment C resulted in a significantly higher  $a^*$  value (redness) and a significantly lower  $b^*$  value (yellowness) than other treatments ( $P < 0.05$ ). Since the  $a^*$  value of treatment C samples was higher than cut-off point at 4.0 [2], it implied that the samples were severely pink. In addition, it was found that 83% of samples in treatment C contained pink color defect, while none of the samples from treatment A and B contained pink color defect. By comparing visual inspection result (qualitative) to color measurement (quantitative) results, it was found that the  $a^*$  and  $b^*$  values could represent the pink color appearance in the cooked chicken drumstick better than the  $L^*$  value. The cooked chicken drumstick could be considered as pink color defect when  $a^* > 4.0$  and  $b^* < 14.0$ .

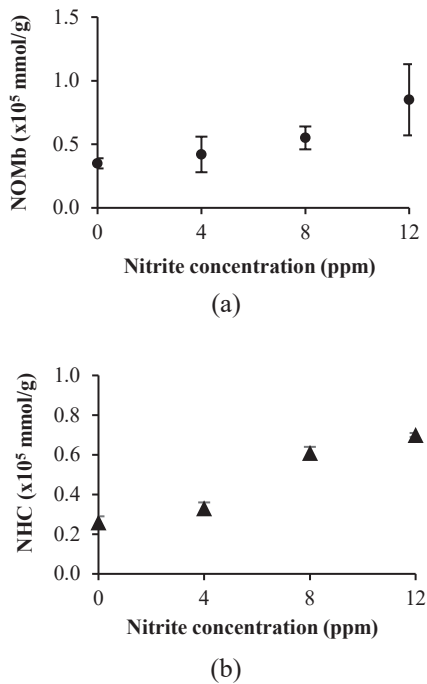
**Table 2:** Color parameters and % pink color defect obtained from different treatments

Treatment	Color Parameters of Steamed Drumstick			Pink Color Defect (%)
	$L^*$ value <sup>ns</sup>	$a^*$ value	$b^*$ value	
A	67.05 $\pm$ 4.06	3.40 <sup>a</sup> $\pm$ 1.30	14.69 <sup>a</sup> $\pm$ 0.70	0
B	67.30 $\pm$ 5.51	3.54 <sup>a</sup> $\pm$ 1.31	15.24 <sup>a</sup> $\pm$ 1.00	0
C	66.14 $\pm$ 3.92	5.97 <sup>b</sup> $\pm$ 0.64	12.96 <sup>b</sup> $\pm$ 0.88	83

Means with different superscripts in the same column differ significantly ( $P < 0.05$ ).

ns – means are not significantly different.

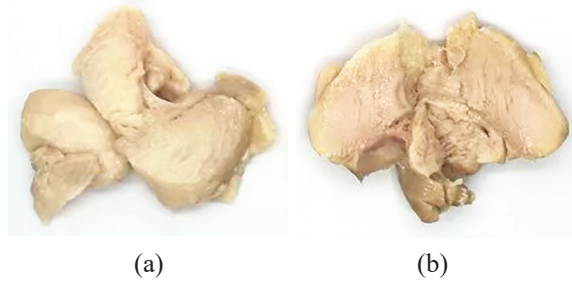
These results indicated that an addition of nitrates to a soy sauce-based marinade (treatment B) had less contribution to the pink color defect formation than the addition of nitrites (treatment C). Comparing between treatment B and C, the NHC content increased almost 3 folds when 8 ppm of nitrite was presented in the marinade formulation (treatment C). These findings corresponded to the fact that nitrates have to be firstly reduced to nitrites in order to form complex structure with myoglobin and form NOMb [18].



**Figure 3:** Effects of nitrite concentration on (a) NOMb and (b) NHC contents in steamed chicken drumstick.

### 3.2 Effect of nitrite content on pink color development in chicken drumsticks

From the previous section, the results implied that nitrite contamination in ginger played an important role in pinking appearance of steamed chicken drumstick. To determine the maximum limit of nitrite contamination to avoid pink color defect problem, sodium nitrite solution at different concentrations (i.e. 0, 4, 8, and 12 ppm) were used as marinades. Effects of nitrite concentration on NOMb and NHC found in steamed chicken drumsticks were illustrated in Figure 3. It seemed that the higher the nitrite concentration, the higher the NOMb and NHC contents found in the samples. Although the nitrite concentration had no significant effect on NOMb content ( $P > 0.05$ ) [Figure 3 (a)], increasing the nitrite content from 4 to 8 ppm significantly increased the NHC content in the steamed chicken drumstick [Figure 3(b)]. Besides a significant increase in NHC content, marinating chicken drumstick in 8 and 12 ppm nitrite solutions resulted in a noticeable pink color defect in the steamed samples as shown in Figure 4 (b).



**Figure 4:** Appearances of steamed chicken drumstick subjected to different nitrite concentrations (a) 0 and 4 ppm (normal) and (b) 8 and 12 ppm (pink color defect).

**Table 3:** Effect of different nitrite concentrations on color parameters and % pink color defect

Nitrite Content (ppm)	Color Parameters of Steamed Drumstick			Pink Color Defect (%)
	$L^*$ value <sup>ns</sup>	$a^*$ value <sup>ns</sup>	$b^*$ value <sup>ns</sup>	
0	66.68 + 1.76	3.69 + 1.06	14.41 + 1.01	0
4	67.38 + 1.83	3.58 + 0.62	14.54 + 0.46	0
8	66.70 + 2.31	4.19 + 0.33	13.39 + 0.32	50
12	66.89 + 0.06	4.75 + 0.38	13.99 + 0.40	84

ns – means are not significantly different.

Considering the color parameters (Table 3), although there was no significant difference in any color parameters when the nitrite concentration increased from 0 to 12 ppm, pink color defect was observed when 8 and 12 ppm nitrite solutions were applied. The visual inspection result agreed well with the  $a^*$  value results in which  $a^*$  values more than 4.0 were obtained in 8 and 12 ppm treatments. Moreover, the percentage of pink color defect also increased as the nitrite concentration increased.

From this study, the nitrite content required to induce a noticeable pink color in chicken drumstick was 1.6 ppm (or 8 ppm in the marinade formulation used in this study) which was comparable to the suggestion in the previous study [11]. They reported that the minimum sodium nitrate for pinking was 1 and 2 ppm for chicken breast and turkey breast, respectively. Since the drumstick has darker background color than the breast, more concentration of nitrite was required to form enough NHC or pink pigment to overcome the background color of the drumstick meat. Thus, the critical nitrite contamination level in chicken drumstick was 4 ppm. Above this critical

limit the probability of pink color defect occurrence could be higher.

#### 4 Conclusions

This study illustrated effects of nitrate and nitrite contamination in ginger paste used in chicken drumstick marination on pigment contents, color and appearance of steamed chicken. For soy sauce-based marinade with ginger paste, the nitrite contamination in ginger paste used in marinade preparation is a key factor contributing to pink color defect formation in steamed chicken drumstick. When 20% marinade (based on raw meat weight) were used, the nitrite concentration in the marinade should be equal or less than 4 ppm (or 0.8 ppm in chicken drumstick) to avoid an occurrence of pink color defect in chicken drumstick. For quality control purpose, the ginger paste should contain no more than 18 ppm of nitrite. Therefore, it is crucial to control the amount of nitrite contamination in ginger paste used in marinade to reduce the risk of pink color defect occurrence.

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