# THE ADDITION OF NITRITE TO PROCESSED MEATS BY PLASMA-TREATED WATER

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Abstract – The aim of this study was to investigate the availability of plasma-treated water (PTW) as nitrite source in curing process of processed meat. PTW contained 50 ppm of nitrite after plasma treatment of distilled water for 60 min. To evaluate the effect of PTW on the generation of curing color, meat batters were manufactured depending on the each formula of three treatments (control, meat batter cured with no nitrite source; PTW, meat batter cured with PTW; Sodium nitrite, meat batter cured with sodium nitrite). After cooking of meat batter, there was no significant difference in the  $L^*$  and  $b^*$  values of cooked meat batter among treatments. The  $a^*$ values of cooked meat batter with PTW was significantly increased compared with that of control although the  $a^*$  values of cooked meat batter with PTW was lower than that of cooked meat batter with sodium nitrite (p < 0.05). Therefore, we conclude that PTW can be used as nitrite source in curing process of processed meat.

Key Words – plasma, nitrite, cored color

# I. INTRODUCTION

In curing process for processed meat, nitrite  $(NO_2^-)$  is used regularly to develop cured color and flavor and to inhibit lipid oxidation and spoilage from microorganisms including Clostridium botulinum [1]. Sodium nitrite is conventionally used as nitrite source to make cured meat product because that is cheap and easy to use. Recently, a use of natural nitrite source has been preferred to the use of synthetic nitrite source such as sodium nitrite as a consequence of that a negative view of synthetic food additive has been increased [2]. Vegetable juices or concentrates including nitrate or nitrite has been generally accepted in processed meat industries and consumers as natural nitrite source [3]. However, some disadvantage was existed in the use of vegetable juices or concentrates as nitrite sources. Vegetable juices or concentrate has own flavor and pigment that could develop an unpleasant flavor and color in cured meat product [4]. In addition, the use of vegetable juices or concentrate results in price increase of cured meat product because of its cost and additional processing step such as incubation for conversion of nitrate to nitrite [5].

Plasma is an ionized gas with charged particles [6]. Plasma treatment is used in variety for sterilization of food and medical instrument, and surface modifications of glass, ceramics and metal materials [6]. In addition, water can be purified by plasma treatment which removes harmful contaminants including microorganisms [7]. Oehmigen *et al.* [8] reported that the interaction of plasma with liquid resulted in the generation of nitrogen species such as nitrate  $(NO_3^-)$  and nitrite  $(NO_2^-)$  as well as reactive oxygen which sterilized the microorganisms. Therefore, we hypothesized that nitrite formed in water by plasma treatment can be used in curing process of processed meat.

The objective of this study is to investigate the nitrite concentration in plasma-treated water (PTW) and the color change of meat batter with plasma-treated water after cooking.

# II. MATERIALS AND METHODS

# Plasma treatment system

As schematically illustrated in Figure 1(a), a plasma device consisting of the powered electrode, ground electrode, and a 0.6 mm-thick alumina plate that was placed between electrodes was used for generating surface dielectric barrier discharge (SDBD). Due to the high concentration of gas-phase oxidants produced at the discharge layer, metallic parts are easily oxidized and induce the change of discharge properties. For



Figure 1. Schematic drawing of plasma apparatus (a), real images of ground electrode (b), and ultra-violet absorption spectrum (c)

this reason, in order to prevent the oxidation of electrode due to the presence of high oxidant species, ground electrode which directly contacts with the discharge was made of a nickel-chromium alloy. A bipolar square waveform with 15 kHz was applied to powered electrode. Total discharge area is 20 cm<sup>2</sup> while the average power is 3.14 W and the peak power is 200 W. Figure 1(b) presents real images of the ground electrode involving rounded square patterns with 3 mm  $\times$  3 mm size. Air surface discharge was generated at the opened area in the face where the ground electrode installed. To produce plasma-treated water, 100 mL distilled water was exposed remotely by SDBD in atmospheric air for 60 min.

#### Quantitation of nitrite concentration

The concentration of nitrite  $(NO_2^-)$  and nitrate  $(NO_3^-)$  dissolved in PTW were measured by using an UV-visible absorption system consisting of the continuum light source (ISS-UV-VIS, Ocean optics Inc., Dunedin, FL., USA), quartz cuvette (CV-Q-10, Ocean optics Inc.) having 10 mm of pathlength, UV-visible optical fiber (QP400-2-SR, Ocean optics Inc.), and spectrometer (MAYA2000 Pro, Ocean optics Inc.). In order to obtain the absorption coefficient of nitrite in the UV range, the standard solutions of nitrite having 100-1000 ppm of the nitrite concentration was prepared by dissolving NaNO<sub>2</sub> (Sigma-Aldrich Co., St. Louis, MO., USA) in distilled water. The absorption spectra of nitrite and nitrate show two distinct

regions, a strong band caused by  $\pi \rightarrow \pi^*$ transition near 200 nm and a weak band by  $n \rightarrow \pi^*$  transition at 270-400 nm [9]. The deconvolution of overlapping absorption bands of nitrite and nitrate at 200 nm is difficult to do, and the high absorption coefficients of both ions at 200 nm are not suitable in this experiment, whereas absorption bands at 270-400 nm are appropriate. Thus, the concentration of nitrite and nitrate were quantified by monitoring the absorption spectra in the wavelength range of 270-400 nm as seen in Figure 1(c).

#### Manufacture of meat batter

Pork hind leg meat and back fat obtained from a commercial butcher's (Daejeon, Korea). Meat was trimmed free of visible fat and connective tissue and then ground using meat grinder with 6 mm plate. Ground meat was mixed with back fat, cooled water, and additives in bowl cutter depending on the each formula of three treatments in Table 1 (control, meat batter cured with no nitrite source; PTW, meat batter cured with PTW; Sodium nitrite, meat batter cured with sodium nitrite). Meat batter was prepared for each treatment per each trials in three trials and vacuum-packaged with dividing packs in low-density into three а polyethylene/nylon vacuum bags and then cooked in water-bath at 80°C for 30 min until internal temperature of cooked meat batter reached 75°C.

Table 1. Formulations for manufacturing meat batter

Ingredient	Control	Sodium nitrite	PTW
Pork hind leg meat	600 g	600 g	600 g
Pork back fat	200 g	200 g	200 g
Ice water	200 g	200 g	-
PTW	-	-	200 g
Sodium chloride	12 g	12 g	12 g
Sodium pyrophosphate	2 g	2 g	2 g
L-ascorbic acid	0.5 g	0.5 g	0.5 g
Sodium nitrite	-	0.1 g	-

#### Instrumental color measurements

The lightness  $(L^*)$ , redness  $(a^*)$ , and yellowness  $(b^*)$  of the sausage were measured using a spectrophotometer (CR-300; Minolta Inc.,

Tokyo, Japan). Measurements were taken perpendicularly to the surface of cooked meat batter with 30 mm diameter of illumination area at 3 different locations per sample.



Figure 2. Ultra-violet absorption spectrum (a) and nitrite concentration (b) of plasma-treated water for 10, 20, 40, and 60 min

### Statistical analysis

Data were analyzed using the PROC GLM procedure of SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) in a randomized complete block design (trial as a block). The experimental unit was the cooked meat batter. The statistical model for instrumental color included the effect of nitrite source. Specific comparisons were performed by Tukey's multiple range test when the main effect was significant. Results are reported as least square mean values and standard error of the least square means (SEM). Statistical significance was considered at P < 0.05.

## III. RESULTS AND DISCUSSION

#### Nitrite concentration in PTW

Increase of plasma treatment time resulted in the increase of nitrite concentration in PTW (Fig 2). After 60 min of plasma treatment, 50 ppm of nitrite was generated in PTW. In addition to charged particles, many reactive neutral species are produced in the discharge layer near the ground electrode. Atmospheric nitrogen and oxygen molecules are dissociated by energetic electrons in the discharge layer mostly forming nitrogen oxides (e.g., NO<sub>2</sub>, N<sub>2</sub>O<sub>3</sub>, and  $N_2O_5$ ) in the gas phase through several reaction pathways [10]. Charged particles have a short lifetime (under  $10^{-6}$  s) and are not able to diffuse over significant distances in aqueous solution. On the other hand, neutral species such as ozone, nitrogen oxides, and nitric acid have greater lifetimes and are able to participate in chemical reactions at the gas-liquid interface. Dissolved NO<sub>2</sub>, NO<sub>3</sub>, N<sub>2</sub>O<sub>4</sub>, and N<sub>2</sub>O<sub>3</sub> react irreversibly with H<sub>2</sub>O to form nitric and nitrous acid via the following reactions; nitric oxide (NO) is sparingly soluble in water [11, 12]:

 $2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \qquad (1)$ 

$$N_2O_4 + H_2O \to HNO_2 + HNO_3 \qquad (2)$$

$$N_2 O_3 + H_2 O \to 2HNO_2 \tag{3}$$

$$HNO_2 \rightleftharpoons H^+ + NO_2^- \tag{4}$$

$$HNO_3 \rightleftharpoons H^+ + NO_3^- \tag{5}$$

#### Instrumental color of cooked meat batter

The  $L^*$  and  $b^*$  values of cooked meat batter was not significantly influenced by addition of nitrite regardless nitrite sources compared with those of control (Table 2). This result was similar with that of previous study. Horsch et al. [5] found that the  $L^*$  value was not different between non-cured ham and cured ham with sodium nitrite. As expected, the  $a^*$  values of control was significantly lower than that of cooked meat batter cured with sodium nitrite or PTW (p < 0.05). The increase of  $a^*$  values in cured meat product with nitrite has been found in various studies regardless of product type such as sausage and ham [5, 13]. Haldane [14] elucidated that the addition of nitrite to meat batter resulted in the generation of nitric oxide, and then to form nitrosylmyoglobin which generated bright pink color of cured meat product after cooking. The  $a^*$  values of cooked meat batter with PTW was significantly lower than that of cooked meat batter with sodium nitrite (P < 0.05). This result was cause by the low concentration of nitrite in cooked meat batter with PTW compared to that in cooked meat batter with sodium nitrite.

Table 2.	Formulat	ions for manu	ıfacturing	meat batter
	Control	Sodium	PTW	$SEM^1$

		nitrite		
$L^*$	67.9	67.0	67.5	0.43
<i>a</i> *	2.0 <sup>c</sup>	10.4 <sup>a</sup>	7.8 <sup>b</sup>	0.29
$b^*$	10.4	9.0	9.6	0.43

<sup>1</sup>Standard errors of mean

<sup>a-c</sup>Different letters within same row differ significantly (P < 0.05).

The PTW used in the present study included 50 ppm of nitrite. So, the added amount of nitrite in meat batter by PTW was 0.001% while that in meat batter by sodium nitrite was 0.007%. Previous study reported the increase of  $a^*$  values in cured ham as a consequence of the increase of nitrite concentration [5].

# **IV. CONCLUSION**

Based on the result in the present study, the cured color of cooked meat batter can be generated by addition of PTW. Therefore, it is possible to use PTW as nitrite source. However, plasma system has to be improved to obtain high amount of nitrite in water. In addition, the distinctive classification of PTW is required because PTW is neither a synthetic nitrite source nor a natural nitrite source. Previous study reported that plasma treatment was one of water purification methods [7]. So, it seems that PTW can be defined as purified water. Therefore, we conclude that PTW can be used as nitrite source in curing process of processed meat.

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