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FULL PAPER

PLASMA PROCESSES AND POLYMERS

An innovative curing process with plasma-treated water for production of loin ham and for its quality and safety

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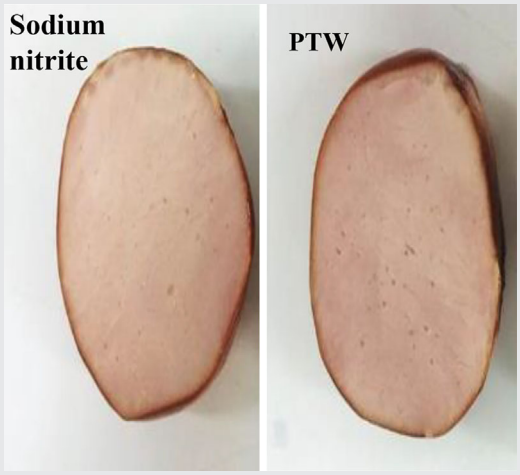
The purpose of the present study is to evaluate the potential of plasma-treated water (PTW) as an alternative to synthetic sodium nitrite in loin ham manufacturing. Two brine solutions made of sodium nitrite were compared against PTW for curing of loin ham. In comparison to chemical brining PTW resulted in increased redness (*a**-value), while allowing to maintain low residual nitrite content and total bacterial counts. No significant differences were found in yellowness (*b**-value), lightness (*L**-value), and lipid oxidation among the treatment groups. Furthermore, the loin ham manufactured using PTW showed no genotoxicity by Ames test. Therefore, PTW could be considered as an effective and innovative substitute for synthetic nitrite in cured meat manufacturing without compromising on quality changes.



KEYWORDS
atmospheric pressure plasma, curing agent, loin ham, nitrite, plasma-treated water

1 | INTRODUCTION

Plasma-treated water (PTW), which means water subjected to plasma discharge in air, has gained increasing attention in several fields, including disinfectants, preservatives, and fertilizers.[1–3] Various uses of PTW are constantly being suggested owing to its pH and the presence of certain chemical species, especially nitrite (NO_2^-), nitrate (NO_3^-),

and hydrogen peroxide (H_2O_2) when N_2 is present in plasma discharge gas.[2,4,5]

In general, nitrite content is decreased while nitrate content is increased in PTW with increasing post-discharge time. This is because subsequent reactions in PTW result in disproportionation of nitrite ($pK_a = 3.3$) into nitrate under acidic conditions: $3NO_2^- + 3H^+ + H_2O \rightarrow 2NO + NO_3^- + H_3O^+$.[3,6] Nonetheless, some studies revealed that nitrite content is maintained during storage when the source liquid is alkaline-buffered solution prior to production of PTW.[6,7] In other words, PTW with adjusted nitrite content can be produced when needed.

Nitrite, an important chemical species in various industrial areas, is also used in meat products because of the following functions: (i) development of a characteristic red color; (ii) protection against food-poisoning bacteria, including *Clostridium botulinum*; (iii) inhibition of lipid oxidation because of a strong antioxidant activity; and (iv) formation of the characteristic cured meat flavor. Therefore synthetic nitrites, including sodium or potassium nitrite, have been used as nitrite sources in meat products for decades.[8,9]

Increasing number of consumers is avoiding synthetic additives owing to the growing concern about food additives in recent years. Consequently, meat products cured with a natural nitrite source have attracted much attention in the industry.[10–12] Vegetable powders such as celery, lettuce, or beet powders contain 1500–2500 mg nitrate/kg. These powders, along with nitrate-reducing bacterial culture, are commercially used in the manufacturing meat products for the functions of nitrite; however, they are not the best alternatives because the incubation steps to reduce nitrate conversion to nitrite are costly and time-consuming.[10,12] In addition, vegetable powders cannot be used in injected meat products. Since bacterial culture is not soluble in the brine solution, the culture does not distribute well within meat during the injection process and uncured zones occur in the final product.[11]

As an alternative to synthetic nitrite and natural nitrite sources (vegetable powder), PTW with adjusted nitrite content was suggested for use in emulsion-type sausage.[7] Our previous work[13] proposed that PTW is neither a chemical reagent nor a natural nitrite source but can be classified as purified water containing nitrite. Using PTW in meat product is also referred to Misra and Jo[14] with more details about plasma and food. To extend industrial utilization of PTW, it is necessary to demonstrate suitability of PTW for manufacturing all types of meat products. Compared to that in emulsion-type sausage, emulsifying and mixing processes with meat and other additives are not required in injected meat products. Thus, it is difficult to uniformly cure an injected meat product with good quality.[11] The purpose of the present study was to compare the quality and microbial safety of injected loin ham, cured with sodium nitrite or PTW. In addition, genotoxicological safety of the products was evaluated.

2 | EXPERIMENTAL SECTION

2.1 | Product manufacture

2.1.1 | Dielectric barrier discharge (DBD) plasma source

The plasma source used in the experiment consists of a powered electrode, ground electrode, and a dielectric plate between the two electrodes (Figure 1). All materials in plasma source and plasma generation conditions were same as those used in our previous study.[7] A bipolar square-waveform voltage at 15 kHz was applied to the powered electrode while the other electrode was grounded. Then, an ambient air discharge was generated at the surface of the electrode. The distance between the ground electrode and liquid surface was 5 cm. In order to obtain visible emission spectrum of the DBD plasma, optical fiber was used near the plasma discharge and recorded using a spectrometer (MAYA2000 Pro, Ocean Optics, Inc., FL, USA).

2.1.2 | Preparation of PTW

To produce PTW, distilled water (500 mL, pH 6.5) containing 1% sodium pyrophosphate (w/v) was treated with the DBD plasma for 2 h. The sodium pyrophosphate was added to

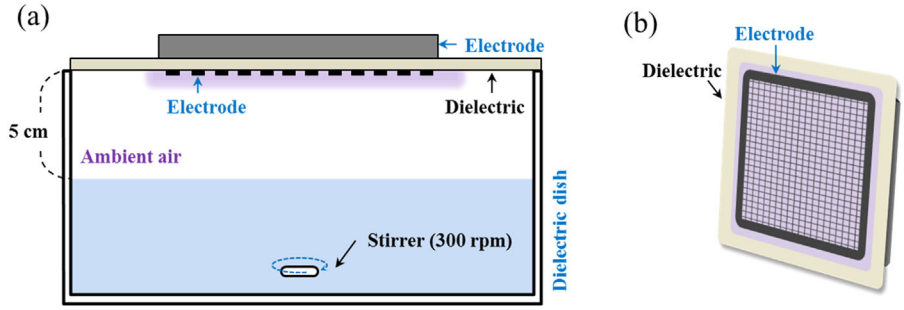


FIGURE 1 Schematic drawing of the full experimental system for the generation of PTW (a), and detailed illustration of DBD actuator (b)

prevent the decrease of pH in PTW because the amount of nitrite ion decreased in acidic PTW. Prepared PTW was used in the next day (approximately 24 h later) to produce loin ham.

The absorption spectra of nitrite and nitrate show two distinct regions.[15] Thus, nitrite and nitrate content in PTW was measured by monitoring the absorption in the wavelength range from 270 to 400 nm as described previously.[7] UV–visible absorption system consisting of the continuum light source (ISS-UV–VIS, Ocean optics Inc., Florida, USA), spectrometer (MAYA2000 Pro, Ocean optics Inc.), and quartz cuvette (CV-Q-10, Ocean optics Inc.) was used in order to obtain the absorption spectra.

The pH values of PTW was measured using a pH meter (SevenGo, Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

2.1.3 | Production of loin ham

Loin ham was produced using raw pork loin (*M. Longissimus dorsi*) obtained from a commercial butcher 2 days postmortem. Two brine solutions were designed to obtain the following concentrations of ingredients, % by total injected weight (raw meat + added solution), in the injected meat: (i) sodium nitrite treatment; sodium nitrite 0.01, sodium pyrophosphate 0.20, water 23.02, sodium chloride 1.07, L-ascorbic acid 0.05, beef-flavored seasoning 1.07, white sugar 1.00, egg white 2.15; (ii) PTW treatment; PTW 20.00, water 3.23, sodium chloride 1.07, L-ascorbic acid 0.05, beef-flavored seasoning 1.07, white sugar 1.00, egg white 2.15. The concentration of nitrite ion in both solutions was maintained at 70 mg kg^{-1}. A multi-needle brine injector (HPI-236, Hyupjin Machine, Co., Ansan, Korea) was used to inject the brine solution to pork loin. Then, the injected pork loins were tumbled for 48 h at 4 °C and smoked until internal temperature of loin ham reached 70 °C. Visual appearances of final products are shown in Figure 2. Each loin ham sample was vacuum-packaged and stored at refrigerator temperature (4 °C). The quality and microbial safety of loin ham samples were analyzed after 0, 1, and 2 weeks of storage, except for nitrosoheme pigment analysis and mutagenicity assay.

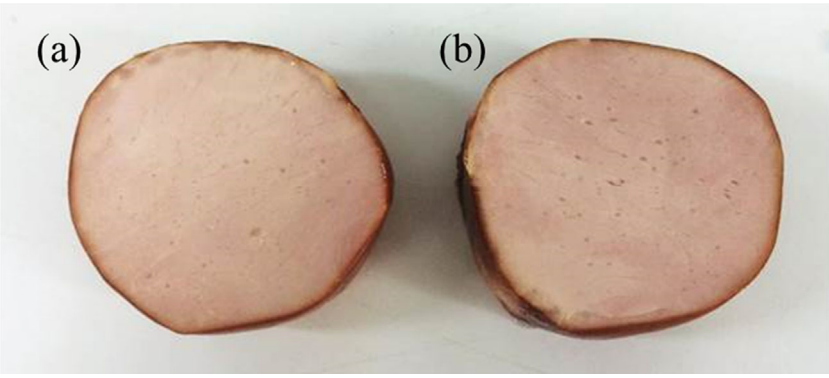


FIGURE 2 Visual appearance of loin ham cured with sodium nitrite (a) and PTW (b)

2.2 | Quality properties

2.2.1 | Color measurements

Surface color of loin ham were conducted on a colorimeter (CR-5, Minolta Camera Co., Osaka, Japan). The instrument was calibrated with a standard black-and-white plate before analysis. Next, L^*, a^*, and b^* measurements were taken at a random location in each sample. A more appropriate measure of color was obtained from the chroma $\left(C = \sqrt{a^2 + b^2}\right)$ and hue ($H = \tan^{-1} b/a$) which were calculated from the a^*, and b^*-values.

Using the L^*-value, lightness or darkness of the sample can be determined where 100 is white, and 0 is black. The a^*-value extends from green ($-a$) to red ($+a$) and the b^*-value from blue ($-b$) to yellow ($+b$). Chroma (saturation index) refers vivid or dull color and is proportional to its intensity. Hue is an angle in a color wheel which is used for color description. An angle of 0° (or 360°) represents red hue, whereas angles of 90°, 180°, and 270° represent yellow, green, and blue hue, respectively.[16]

2.2.2 | Absorption spectra of acetone extracts

After manufacturing, the loin ham (10 g) was placed in a brown bottle. Then, acetone (40 mL) and distilled water (3 mL) were added and mixed for 5 min. The mixture was filtered through a Whatman filter paper no. 1 (Whatman International Ltd., Springfield Mill, Kent, United Kingdom), and absorption scans of the solution were conducted from 380 to 600 nm at 1-nm increments, using a Model X-ma 3100 spectrophotometer (Human Co., Ltd., Seoul, Korea).

2.2.3 | Residual nitrite content

This characteristic of loin ham was determined according to AOAC method 973.31.[17]

2.2.4 | Total aerobic bacterial counts

A loin ham sample (5 g) was taken aseptically from each treatment group, transferred to a sterile plastic pouch, and homogenized for 2 min at room temperature with 45 mL of sterile saline, using a stomacher (BagMixer 400, Interscience Ind., St. Nom, France). Appropriate dilutions of the samples were

prepared in sterile saline and plated onto tryptic soy agar (Difco Laboratories, Detroit, MI, USA). The agar plates were incubated at 37 °C for 48 h under aerobic conditions. The results were expressed as log numbers of colony-forming units per gram (Log CFU/g).

2.2.5 | Peroxide value (POV)

First, lipid extraction was conducted according to Folch's extraction method.[18] The extracted lipid sample was placed into a 100-mL Erlenmeyer flask, and we added 35 mL of an acetic acid/chloroform (3:2) mixture and 0.5 mL of a saturated potassium iodide solution. The mixture was kept in the dark for 5 min, after which distilled water (75 mL) was added. The solution was titrated with a 0.005 N sodium thiosulfate ($Na_2S_2O_3$) solution, using a 1% starch solution (2.5 mL) as an indicator. The POV was calculated by means of the following formula:

$$\mathrm{POV(meq/kg)} = [(S - B) \times F \times 0.01]/SW \times 100$$

where S is the titration volume (mL) of 0.005 N $Na_2S_2O_3$ in the samples, B is the titration volume (mL) of 0.005 N $Na_2S_2O_3$ in the blank, F is the factor of the 0.005 N $Na_2S_2O_3$ solution, and SW is the sample weight (g).

2.3 | Mutagenicity assay

This assay was performed on ethanolic extracts of loin ham samples at time point zero (before storage) and PTW, respectively. Loin ham sample (100 g) was chopped and mixed with 900 mL of 70% ethanol for 8 h at 25 °C. The extracts were filtered using Whatman filter paper no. 4 (Whatman International, Ltd.). After that ethanol was removed from the samples using a vacuum evaporator (Rotary Vacuum Evaporator N-11 Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The extracts were lyophilized (Freeze dry system, Labconco, FreeZone 18, Kansas City, KS, USA) after being frozen and were kept in a freezer (−70 °C) before use. On the other hand, PTW was used without further process.

Salmonella mutagenicity assay uses *Salmonella* strains with preexisting mutations that leave the bacteria unable to synthesize the required histidine. Therefore, histidine-dependent *Salmonella* Typhimurium strains TA98 (hisD3052/*rfa*/Δ*uvr*B/pKM101) and TA100 (hisG46/*rfa*/Δ*uvr*B/pKM101) were purchased from the Korea Institute of Toxicology KIT, Daejeon, Korea and used. When a mutagen is added to the agar plate with the strains, newly mutated cells can grow in the absence of histidine and form colonies.[17,19] The potential mutagenic effects of the loin ham samples were assessed by the Ames test according to Maron and Ames[19] and Lee et al.[20]

2.4 | Statistical analysis

One-way analysis of variance with a completely randomized design was performed using the procedure of the general linear model (GLM). Significance of differences among mean values was determined by Duncan's multiple-comparison tests in the SAS software, Release 9.4 (SAS Institute Inc., Cary, NC, USA), with the confidence level of $P < 0.05$. Mean values and standard deviations were presented. All the experimental procedures were conducted in triplicate with two observation numbers.

3 | RESULTS AND DISCUSSION

3.1 | Emission spectrum of DBD plasma and chemical properties of PTW

In the DBD plasma discharge, NO_g, N_2 (second positive system), and N_2^+ (first negative system) molecular spectra were detected (Figure 3). The majority of intense peaks were near the ultraviolet (UV) region (300–400 nm) in terms of emission. Hydroxyl radicals were expected to occur in the plasma used here but may not have been detected owing to their short lifetime. Generally, the main radicals present in plasma discharge are nitric oxide (NO·) and hydroxyl radicals (OH·) when ambient air serves as a working gas.[21] In these conditions, the formation of nitrogen oxides and ozone are expected. Then, reactions of these molecules from the plasma with the aqueous liquid can result in generation of nitrites, nitrate, and hydrogen peroxide.[22] Initial concentration of both nitrite and nitrate in untreated water (1% sodium pyrophosphate in distilled water, w/v) was 0 ppm. After 2 h of DBD plasma treatment, the nitrite and nitrate content of PTW reached 782 and 358 ppm, respectively. The pH of PTW was

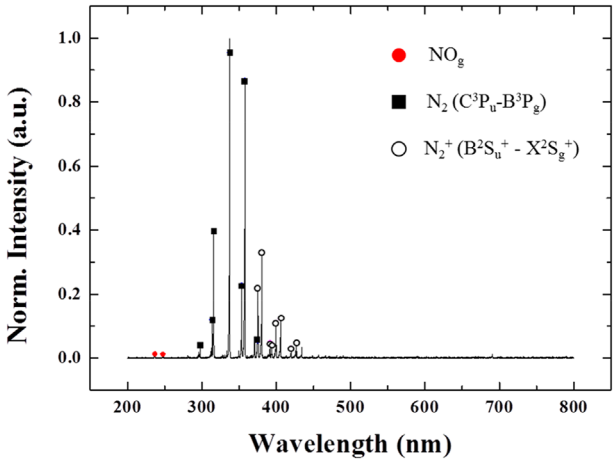


FIGURE 3 An emission spectrum of the DBD plasma. NO, N_2, and N_2^+ molecular peaks were generated because ambient air was used

changed from 10.19 to 9.01 after DBD plasma treatment for 2 h.

3.2 | Surface color and nitrosoheme pigment content

Ham cured with PTW showed no significant difference from sodium nitrite-treated ham in the *L**, *b**, and chroma values. Meanwhile, the *a**-value was greater and hue angle was lower in the PTW-treated samples compared to those of sodium nitrite-treated ones during storage (Table 1). In other words, the ham manufactured by PTW showed higher redness (associated with high *a**-value and low hue angle) compared to that of sodium nitrite.

Generally, cured meat product showed characteristic red color due to the reaction of myoglobin and nitric oxide.[8] Myoglobin is the major contributor to meat color and is composed of an iron, heme-group, and globin protein. Iron in myoglobin is ligated with the four nitrogen atoms of the heme group and one nitrogen atom of globin protein. The last, sixth, position of the iron remains available to bind electronegative atoms of various ligands. Therefore, oxygen, carbon monoxide, nitric oxide, and other molecules can bind to the iron in myoglobin, where binding of different ligands affords different meat colors.[10,11]

In a meat product, nitrous acid (HNO_2) derived from nitrite can form nitrous acid anhydride, which is in equilibrium with nitric dioxide and nitric oxide. Then, nitric oxide can react with iron in myoglobin and form nitroso-myoglobin, which is responsible for the distinct red-cured color.[8,9] Even though the protein moiety of nitroso-myoglobin is denatured by heat treatment, the nitroso-myochromogen (nitroso-heme pigment) persists and shows a stable red color.[8]

To identify different redness intensity levels, absorption spectra of acetone extracts from different loin ham samples were examined (Figure 4). Maximal absorption was obtained approximately at 540 and 574 nm, corresponding to the pattern of the nitroso-heme pigment.[23] In addition, PTW treatment yielded higher maximal absorbance, which means higher nitroso-heme pigment content in comparison with that yielded by the sodium nitrite treatment. High redness of ham cured with PTW may be due to the nitroso-heme pigment content.

3.3 | Residual nitrite content

This parameter was lower in the loin ham made with PTW than in the sodium nitrite-treated ham every week during storage, even though the same amount of nitrite ion was added initially (Table 2). Honikel[8] reported that residual nitrite content in a meat product decreases when more nitrite is converted to nitrogen oxide (NO). Next, increased amounts of nitrogen oxides react with myoglobin and form more of the

TABLE 1 Surface color of ham cured with different nitrite sources

	Storage (weeks)		
Treatment	**0**	**1**	**2**
*L**-value			
Sodium nitrite	$71.03 \pm 0.70^{1)}$	71.00 ± 0.65	71.08 ± 0.47^{y}
PTW	71.51 ± 0.90^{ab}	70.55 ± 0.75^{b}	73.23 ± 1.15^{ax}
*a**-value			
Sodium nitrite	6.65 ± 0.15^{y}	6.50 ± 0.22^{y}	6.35 ± 0.35^{y}
PTW	7.28 ± 0.13^{x}	7.40 ± 0.40^{x}	7.10 ± 0.23^{x}
*b**-value			
Sodium nitrite	9.64 ± 1.78	9.57 ± 0.14	9.57 ± 0.15
PTW	9.50 ± 0.50	9.64 ± 0.23	9.39 ± 0.05
Chroma			
Sodium nitrite	11.71 ± 0.17	11.57 ± 0.23	11.48 ± 0.26
PTW	11.97 ± 0.47	12.15 ± 0.38	11.77 ± 0.15
Hue			
Sodium nitrite	55.42 ± 0.79^{x}	55.82 ± 0.64^{x}	56.47 ± 1.42^{x}
PTW	52.50 ± 0.96^{y}	52.51 ± 1.20^{y}	52.90 ± 0.84^{y}

[1)]Values are the mean ± SD ($P < 0.05$).

[a–c]Values with different letters within the same row differ significantly ($P < 0.05$).

[x,y]Different letters within the same column indicate that the values differ significantly ($P < 0.05$).

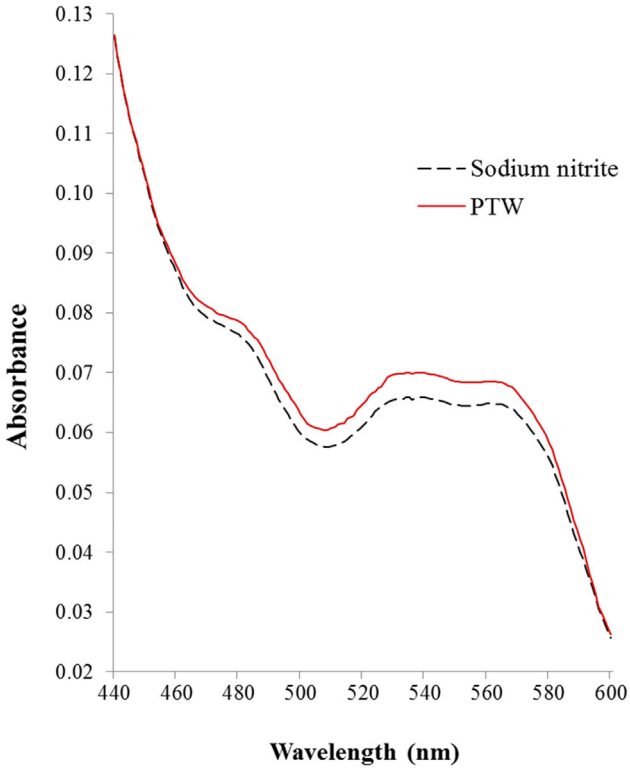


FIGURE 4 Absorption spectra of acetone extracts of loin ham after manufacturing

nitroso-heme pigment. In the present study, a larger amount of the nitroso-heme pigment was actually produced in the PTW-treated ham compared to that in the sodium nitrite-treated ham (Figure 3). According to the results, nitrite may be more easily reduced to nitric oxide with PTW treatment than with sodium nitrite treatment. Jung et al.[7] reported that either the conversion of nitrite to nitric oxide or the reaction of nitrite with ascorbic acid (one of added reductants) is rapid when added nitrite is dissolved in a solution (PTW) rather than in the solid state (sodium nitrite or celery powder). However, the residual nitrite content was lower in the ham cured with PTW than that with sodium nitrite, although the sodium nitrite was dissolved in water and used as same form as PTW.

Some studies have also shown that irradiation reduces residual nitrite content of meat products.[24] Simie[25] reported that nitrite downregulation by irradiation is due to its reaction with the hydroxyl radical resulting from the radiolysis of water. The hydroxyl radical can also be present in PTW, but it is not known whether it survives until the use in a meat product owing to the short lifetime (approximately 10^{-9} s).[4,26] Further in-depth research is necessary to elucidate the exact reason for the lower residual nitrite content in ham samples subjected to PTW treatment.

Residual nitrite content of loin ham samples in both treatment groups decreased after 2 weeks of storage (Table 2). Alahakoon et al.[10] showed that residual nitrite content in meat products gradually declines during storage because of light- or oxidation-induced fading. Meanwhile, Ahn et al.[24] demonstrated that residual ascorbic acid converts nitrite, resulting in a decrease in residual nitrite content in a meat product during storage.

The safety of a cured meat product is a significant issue for two reasons that are related to residual nitrite.[8,11] First, nitrite is an effective antimicrobial agent, particularly for preventing toxin production by *C. botulinum*. After addition of nitrite, nitric oxide can react with iron-sulfur proteins (in bacteria), which are necessary for energy production.[10] For this reason, a proper amount of residual nitrite should be maintained in a meat product for antibotulism protection. In contrast, a high residual nitrite content is a known health risk factor because of potential formation of carcinogenic

TABLE 2 Physicochemical and microbiological properties of loin ham cured with different nitrite sources

	Storage (weeks)		
Treatment	**0**	**1**	**2**
Residual nitrite (ppm)			
Sodium nitrite	$24.68 \pm 1.94^{ax,1)}$	23.30 ± 0.77^{ax}	20.38 ± 1.55^{bx}
PTW	14.96 ± 0.65^{ay}	13.93 ± 0.64^{aby}	10.36 ± 1.20^{by}
Total aerobic bacteria (Log CFU/g)			
Sodium nitrite	4.21 ± 0.11^{cx}	6.25 ± 0.04^{b}	6.68 ± 0.11^{a}
PTW	3.88 ± 0.07^{cy}	6.14 ± 0.09^{b}	6.52 ± 0.09^{a}
Peroxide value (meq/kg)			
Sodium nitrite	1.17 ± 0.28^{b}	1.80 ± 0.32^{a}	1.55 ± 0.03^{ab}
PTW	1.27 ± 0.17^{b}	1.96 ± 0.10^{a}	1.82 ± 0.14^{a}

[1)]Values are the mean ± SD ($P < 0.05$).

[a–c]Values with different letters within the same row differ significantly ($P < 0.05$).

[x,y]Different letters within the same column indicate that the values differ significantly ($P < 0.05$).

nitrosamines.[24,27] The nitrosamines can be produced from secondary amines with nitrite in a specific condition such as high temperature (>130 °C) and acidic pH. A number of consumers are interested in lower residual nitrite content, despite the low probability of occurrence of nitrosamines in meat products.[8] In both regards, residual nitrite content should be carefully controlled to ensure product safety.

3.4 | Total aerobic bacteria

The mechanisms of interaction between PTW and bacteria are not fully understood. Nonetheless, most authors agree that the bactericidal effects of PTW are predominantly due to hydrogen peroxide, nitrites, nitrates, peroxy-nitrites, and pH changes.[6] Traylor et al.[28] hypothesized that among these antimicrobial reagents, long-lived secondary products such as hydrogen peroxide, nitrite, or nitrate are responsible for the extended antimicrobial effects of PTW. Therefore, an alkaline-buffered solution was treated by plasma, and the resulting PTW contains high concentrations of nitrite and hydrogen peroxide.[1] On the other hand, only a small reduction in the number of *Escherichia coli* cells (<0.5 Log CFU) was achieved by means of PTW.[1] Likewise, a weak antimicrobial effect was observed when PTW made from an alkaline solution was applied to *Hafnia alvei* suspension for up to 30 min.[5]

PTW in the present study was also made from an alkaline solution and showed a weak antimicrobial effect. In Table 2, the initial number of total aerobic-bacteria cells in the PTW-treated ham samples was 0.33 Log CFU/g lower in comparison with sodium nitrite-treated samples. At 1 and 2 weeks of storage, no significant differences were observed in the number of total aerobic-bacteria cells between the two treatments.

3.5 | Lipid oxidation

If free radicals, hydrogen peroxide, or reactive oxygen and nitrogen species exist in PTW used in the present study, they should initiate lipid oxidation in a meat product.[29] In contrast, no significant differences were observed in the peroxide value between PTW- and sodium nitrite-treated samples throughout the entire period of storage. After 1 week of storage, peroxide values in both treatment groups increased (Table 2). Peroxide is formed as a primary product during lipid oxidation.[30]

Lipid oxidation in meat products is prevented by nitric oxide derived from nitrite. This is because nitric oxide can bind to the iron in meat pigments and lower the amount of free iron, which is a potent catalyst of lipid oxidation.[9,10,31] In the present study, more nitric oxide reacted with myoglobin and formed more of the nitrosoheme pigment (Figure 3). Nonetheless, the difference in nitrosoheme pigment content may not be sufficient to detect a significant difference in lipid oxidation between the two treatments during 2 weeks of storage. When emulsion-type sausage was cured with PTW,

TABLE 3 *Salmonella* mutagenicity assay for loin ham cured with different nitrite sources

		Number of revertant colonies (His+) per plate[a]			
Treatment	**Dose (μg/plate)**	**TA98 (−S9)**	**TA98 (+S9)**	**TA100 (−S9)**	**TA100 (+S9)**
Sodium nitrite	188	30 ± 7	39 ± 5	329 ± 47	323 ± 41
	375	22 ± 2	32 ± 6	323 ± 33	365 ± 23
	750	34 ± 3	30 ± 6	385 ± 44	468 ± 4
	1500	32 ± 4	34 ± 4	361 ± 51	341 ± 15
	3000	33 ± 6	29 ± 3	341 ± 65	456 ± 50
PTW	188	16 ± 5	23 ± 9	293 ± 85	282 ± 19
	375	24 ± 12	32 ± 4	317 ± 59	329 ± 61
	750	22 ± 4	33 ± 1	338 ± 72	338 ± 40
	1500	19 ± 6	28 ± 8	291 ± 6	308 ± 6
	3000	20 ± 3	27 ± 7	332 ± 29	346 ± 13
Negative control[b]	EtOH	22 ± 3	21 ± 5	294 ± 13	301 ± 25
Positive control[b]	4-NQO	1108 ± 22			
	2-AA		2214 ± 48		
	SA			902 ± 96	
	2-AA				2423 ± 108

[a]Values are the mean ± SD ($P < 0.05$).
[b]EtOH, 70% ethanol; 4-NQO, 4-nitroquinoline-1-oxide; SA, sodium azide; 2-AA, 2-aminoanthracene.

TABLE 4 *Salmonella* mutagenicity assay for PTW

Sample		Number of revertant colonies (His+) per plate[a]			
		TA98 (−S9)	TA98 (+S9)	TA100 (−S9)	TA100 (+S9)
PTW		18 ± 5	26 ± 3	204 ± 7	266 ± 25
Negative control	Distilled water	17 ± 4	22 ± 4	323 ± 21	338 ± 33
Positive control[b]	4-NQO	1063 ± 14			
	2-AA		2055 ± 95		
	SA			861 ± 88	
	2-AA				2343 ± 112

[a]Values are the mean ± SD ($P < 0.05$).
[b]4-NQO, 4-nitroquinoline-1-oxide; SA, sodium azide; 2-AA, 2-aminoanthracene.

no significant differences in peroxide values were observed in comparison with emulsion-type sausage cured with sodium nitrite during the 28 days of storage.[7]

3.6 | Mutagenicity assay

The Ames *Salmonella* mutagenicity test is a short-term bacterial reverse mutation assay designed to detect a wide range of chemicals that can generate genetic damage and lead to gene mutations.[32] In the mutagenicity assay, a sample being tested is positive for mutagenicity when the number of revertant colonies is higher than that in the negative control.[16] As shown in Table 3, the number of revertants per plate for the sodium nitrite and PTW treatments was almost the same as that in the negative control. In other words, loin ham cured with sodium nitrite or PTW at doses of up to 3000 μg/plate is not mutagenic. The numbers of revertants per plate in positive controls were 20- and 3-fold higher than those in the samples tested, which means that the experiment was performed properly.[17]

PTW used in this study was found to be not genotoxic according to the *Salmonella* mutagenicity assay (Table 4). Addition of PTW to emulsion-type sausage has no mutagenic effect either.[33] As for immune toxicity, Balb/c mice were given free access to sausage cured with PTW; 32 days later, tumor necrosis factor (TNF)-α levels were evaluated. As a result, a TNF-α value less than 10 μg mL^{-1} was detected in mice eating control and treated samples, respectively. This finding indicates that no inflammatory response is triggered in mice consuming sausage cured with PTW.[33]

4 | CONCLUSION

The present study was aimed to see the possibility of PTW as an alternative of synthetic sodium nitrite in processed meat manufacturing such as loin ham. From the results, color-developing capacity is higher and residual nitrite content is lower in the ham treated with PTW than in that treated with sodium nitrite. Genotoxicological safety of the loin ham manufactured with PTW was confirmed by the Ames test. Because there has been no effective substitute for synthetic nitrite in cured meat processing so far, particularly injection type meat products due to solubility, PTW can be considered as a suitable and cost-effective alternative to synthetic nitrite or nitrite-containing vegetable powders for natural curing process.

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REFERENCES

[1] N. N. Misra, O. C. Schlüter, P. J. Cullen, *Cold Plasma in Food and Agriculture: Fundamentals and Applications*, Academic Press, Elsevier (ISBN: 978-0-12-801365-0), **2016**.
[2] R. Ma, G. Wang, Y. Tian, K. Wang, J. Zhang, J. Fang, *J. Hazard. Mater.* **2015**, *300*, 643.
[3] D. P. Park, K. Davis, S. Gilani, C. A. Alonzo, D. Dobrynin, G. Friedman, G. Fridman, *Curr. Appl. Phys.* **2013**, *13*, S19.
[4] R. Burlica, R. G. Grim, K. Y. Shih, D. Balkwill, B. R. Locke, *Plasma Process. Polym.* **2010**, *7*, 640.
[5] M. Naïtali, G. Kamgang-Youbi, J. M. Herry, M. N. Bellon-Fontaine, J. L. Brisset, *Appl. Environ. Microbiol.* **2010**, *76*, 7662.
[6] Z. Machala, B. Tarabova, K. Hensel, E. Spetlikova, L. Sikurova, P. Lukes, *Plasma Process. Polym.* **2013**, *10*, 649.
[7] S. Jung, H. J. Kim, S. Park, H. I. Yong, J. H. Choe, H. J. Jeon, W. Choe, C. Jo, *Meat Sci.* **2015a**, *108*, 132.
[8] K. O. Honikel, *Meat Sci.* **2008**, *78*, 68.
[9] D. K. Parthasarathy, N. S. Bryan, *Meat Sci.* **2012**, *92*, 274.
[10] A. U. Alahakoon, D. D. Jayasena, S. Ramachandra, C. Jo, *Trends Food Sci. Technol.* **2015**, *45*, 37.

[11] J. G. Sebranek, J. Bacus, *Am. Meat Sci. Association White Paper Ser*. **2007**, *1*, 115.
[12] J. G. Sebranek, A. L. Jackson-Davis, K. L. Myers, N. A. Lavieri, *Meat Sci*. **2012**, *92*, 267.
[13] S. Jung, H. J. Kim, S. Park, H. I. Yong, J. H. Choe, H. J. Jeon, W. Choe, C. Jo, *Korean J. Food Sci. An*. **2015b**, *35*, 703.
[14] N. N. Misra, C. Jo, *Trends Food Sci. Technol*. **2017**, *64*, 74.
[15] K. S. Krishnan, A. C. Guha, *Proc. Indian Acad. Sci*. **1934**, *1*, 242.
[16] R. G. McGuire, *HortScience* **1992**, *27*, 1254.
[17] AOAC. Official methods of analysis of AOAC International 16th ed., Virginia, USA, **1995**, AOAC Vols. II. pp. 938–940.
[18] H. Folch, M. Lee, S. G. H. Stanley, *J. Biol. Chem*. **1957**, *226*, 497.
[19] D. M. Maron, B. N. Ames, *Mutat. Res*. **1983**, *113*, 173.
[20] H. Lee, H. I. Yong, H. J. Kim, W. Choe, S. J. Yoo, E. J. Jang, C. Jo, *Food Sci. Biotechnol*. **2016**, *25*, 1189.
[21] S. Shimmura, T. Masumizu, Y. Nakai, K. Urayama, J. Shimazaki, H. Bissen-Miyajima, M. Kohno, K. Tsubota, *Invest. Ophthalmol. Vis. Sci*. **1999**, *40*, 1245.
[22] K. Oehmigen, J. Winter, M. Hähnel, C. Wilke, R. Brandenburg, K. D. Weltmann, T. von Woedtke, *Plasma Process. Polym*. **2011**, *8*, 904.
[23] G. Parolari, L. Gabba, G. Saccani, *Meat Sci*. **2003**, *64*, 483.
[24] H. J. Ahn, C. Jo, J. W. Lee, J. H. Kim, K. H. Kim, M. W. Byun, *J. Agric. Food Chem*. **2003**, *51*, 1249.
[25] M. C. Simie, *Preservation of Food by Ionizing Radiation*, CRC Press, Boca Raton, FL **1983**, vol. 2, p. 8.
[26] J. Aikens, T. A. Dix, *J. Biol. Chem*. **1991**, *266*, 15091.
[27] R. B. Pegg, F. Shahidi, *Nitrite Curing of Meat*, Food and Nutrition Press Inc., Trumbull, CT **2008**.
[28] M. J. Traylor, M. J. Pavlovich, S. Karim, P. Hait, Y. Sakiyama, D. S. Clark, D. B. Graves, *J. Phys. D: Appl. Phys*. **2011**, *44*, 472001.
[29] B. Min, D. U. Ahn, *Food Sci. Biotechnol*. **2005**, *14*, 152.
[30] H. I. Yong, H. Lee, S. Park, J. Park, W. Choe, S. Jung, C. Jo, *Meat Sci*. **2017**, *123*, 151.
[31] J. J. Sindelar, A. L. Milkowski, *Nitric Oxide* **2012**, *26*, 259.
[32] K. Mortelmans, E. Zeiger, *Mutat. Res*. **2000**, *455*, 29.
[33] H. J. Kim, N. Y. Sung, H. I. Yong, H. Kim, Y. Lim, K. H. Ko, C. Yun, C. Jo, *Korean J. Food Sci. An*. **2016**, *36*, 494.

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