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Curing of ground ham by remote infusion of atmospheric non-thermal plasma

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ABSTRACT

This study investigated the changes in the nitrite content and temperature of meat batter with the remote infusion of atmospheric non-thermal plasma (ANP) and the quality properties of ground hams cured by remote infusion of ANP. Remote infusion of ANP for 14.78 min generated 100 mg kg^{-1} of nitrite in 3.2 kg meat batter, and the meat batter temperature increased from 1.8 to 9.2 °C. The residual nitrite content of ground ham cured by the remote infusion of ANP (RANP) was higher than that of ground ham cured with sodium nitrite (SN) at 1 day of storage ($p < 0.05$); however, no difference between RANP and SN was observed after 30 days of storage ($p > 0.05$). The color and malondialdehyde content of ground hams did not differ between RANP and SN during storage ($p > 0.05$). The remote infusion ANP system could be applied to cure meat products without the addition of nitrite sources.

1. Introduction

Plasma is ionized gas containing positive and negative ions, electrons, metastables, atoms, free radicals, and photons, which are generated by supplying energy, such as thermal energy, an energetic beam, or an electric field, to neutral gas (Misra, Yadav, Roopesh, & Jo, 2019). Plasma can be generated at low pressure or atmospheric pressure, and is classified as thermal or non-thermal plasma based on its thermodynamic states (Misra et al., 2018). Among the various plasma technologies, atmospheric non-thermal plasma (ANP) that generates reactive chemical species under atmospheric pressure and ambient gas temperature because of thermodynamic *non-equilibrium* state has received attention for its high applicability in the food industry, as it does not require vacuum equipment or result in severe heat damage to the food quality (Lee et al., 2017; Misra et al., 2018).

The use of ANP as a curing process in the meat industry has recently been reported (Jo, Lee, Lim, Hwang, & Jung, 2018; Jung, Lee, Lim, et al., 2017; Lee et al., 2017; Misra & Jo, 2017). Cured meat products are processed meat products containing nitrites. The addition of nitrites to meat products generates the pink color and flavor associated with curing, and inhibits spoilage and the growth of pathogenic microorganisms including *Clostridium botulinum* during storage (Sebranek, 2009). For these reasons, synthetic nitrites, such as sodium or potassium nitrite, are generally added to manufacture cured meat products. However, synthetic food additives have been gradually rejected by consumers, as consumers increasingly perceive them to have a negative impact on health (Jo, Lee, & Jung, 2018; Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). Plasma generated with the gases containing nitrogen contains reactive nitrogen species. Ercan, Smith, Ji, Brooks, and Joshi (2016) and Jung et al. (2015) found the generation of nitrite in the liquid phase after plasma treatment due to the reactions between the reactive nitrogen species and water molecules. In addition, nitrites can be generated when meat batter is treated with ANP (Jo, Lee, Lim, et al., 2018; Jung, Lee, Lim, et al., 2017; Lee et al., 2018).

Previous studies found a gradual increase of the nitrite content in meat batter with increasing ANP treatment time (Jung, Lee, Lim, et al., 2017; Lee et al., 2018). However, the temperature of the meat batter also gradually increased due to heat absorption from the plasma and plasma discharge source (Jung, Lee, Lim, et al., 2017; Lee et al., 2018). Therefore, the optimum level of nitrite that could be generated in meat

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batter by direct treatment with ANP without exceeding the recommended temperature (< 13 °C) was 42 mg kg^{-1} (Lee et al., 2018; Pegg & Boles, 2014). The regulations regarding added nitrites in cured meat products differ depending on the country and category of meat product. The allowed levels of added nitrites for comminuted products in the European Union and United States are 150 and 156 mg kg^{-1} (as sodium nitrite), respectively (Sebranek, 2009). South Korea limits residual nitrites to less than 70 mg kg^{-1} in all cured meat products. Nitrites can develop a cured color and flavor in cured meat products at levels as low as 10 mg kg^{-1}; however, it is recommended to add as much nitrite as allowed to ensure microbial safety (Jung et al., 2015; Sebranek et al., 2012). Therefore, a method to increase the nitrite level of meat batter up to the allowed level via treatment with ANP without exceeding the recommended meat batter temperature is required for ANP to be adopted as a curing process in meat industry.

ANP can be used for remote treatment. In remote ANP treatment, plasma generated in the plasma discharge source is transferred to the sample via a tube with a pump system (Hertwig et al., 2015). In this system, the plasma is cooled during transportation, and the heat of the plasma discharge source is not transferred to the samples (Hertwig et al., 2015; Jo, Lee, Lim, et al., 2018). Although there is some risk that the reactive species in plasma could disappear before reaching the samples, Hertwig et al. (2015) found that remote plasma treatment achieved a microbicidal effect. Therefore, we hypothesized that remote ANP treatment could generate a nitrite as much as allowed in the meat batter without the severe increase of its temperature. The nitrite content and temperature of the meat batter after remote infusion of ANP were tested, and the quality characteristics of ground hams cured using remote ANP infusion were evaluated.

2. Material and methods

2.1. Properties of the meat batter with the remote infusion of ANP

2.1.1. Preparation of the meat batter

Hind-leg pork and back fat were purchased at a local market (Daejeon, Korea). Visible fat and connective tissue were removed from the meat, which was then ground using a meat grinder (M-12S, Hankook Fugee Industries Co., Ltd., Hwaseong, Korea) with a 6 mm plate. Ground pork (2.6 kg), back fat (0.4 kg), water (0.2 kg), other ingredients (sodium chloride 2%, isolated soy protein 0.3%, and sugar 1%), and sodium pyrophosphate 0.3% were mixed in the food mixer with the remote infusion of ANP.

2.1.2. Remote infusion of ANP

The system for the remote infusion of ANP used in this study is shown in Fig. S1 of the Supplementary data. The plasma discharge source utilized cylindrical dielectric-barrier discharge (DBD) (Plasmapp Co., Ltd., Daejeon, Korea). Ambient air was used as the process gas for the plasma discharge, and the plasma was discharged at 1.5 kW and 60 kHz of power consumption. The plasma generated in the plasma discharge source was delivered to the food mixer through a polytetrafluoroethylene tube (5 mm internal diameter). The cooling path was formed inside the plasma discharge source, and 15 °C water was circulated to cool the plasma discharge source during plasma generation.

The remote ANP treatment of the meat batter was conducted three times (three operations) in the same day at 1 h intervals, and this test was repeated three times (three trials) on different days. Meat batter samples were collected from three areas of the meat batter at 5 min intervals during the infusion of ANP over 30 min, and the nitrite concentrations of the meat batter samples were measured.

2.1.3. Nitrite concentration

The meat batter samples collected during the remote ANP infusion treatment were immediately stored in a −70 °C deep freezer until analysis. The nitrite contents of the meat batters were measured according to AOAC method 973.31 (AOAC, 1990) with modifications. The meat batter (10 g) was thoroughly mixed with 150 mL of hot water (80 °C) in a 250 mL volumetric flask, and then 10 mL of 0.5 M NaOH was added. After mixing, 10 mL of 12% zinc sulfate was added to the flask, and the sample was again thoroughly mixed. The flask was heated for 20 min in a shaking water bath at 80 °C. After cooling in tap water for 10 min, 20 mL of 10% ammonium acetate (pH adjusted to 9.1 with 10% ammonia-water) was added to the flask, and the mixture was diluted to a volume of 200 mL using deionized water. After mixing, the solution was filtered through a Whatman No. 4 filter paper (Whatman, Maidstone, England). A 20 mL of the filtrate (sample solution) was transferred to a 25 mL volumetric flask, followed by the addition of 1 mL of 30 mM sulfanilamide in acid solution (HCl:water, 1:1, v/v) and 1 mL of 5 mM *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resultant solution was diluted to 25 mL with deionized water. The mixed solution was allowed to stand for 20 min to allow complete color development. The absorbance of the solution was measured at 540 nm using a spectrophotometer (DU®530, Beckman Instruments Inc., CA., USA), with distilled water as a reference sample. The nitrite concentration of the meat batter was calculated using a standard curve prepared from absorbance readings of standard solutions of $NaNO_2$ (Sigma-Aldrich Co., St. Louis, MO., USA).

2.1.4. Meat batter temperature

The temperature of the meat batter was monitored during the infusion of ANP for 20 min using an inserted probe connected to a digital thermometer (YF-160A, Koang Yee Enterprise Co., Ltd., Taipei, Taiwan). The temperature of the meat batter was recorded at 5 min intervals

2.2. Quality properties of the ground hams

2.2.1. Manufacture of the ground hams and sample collection

Ground pork (2.6 kg), back fat (0.4 kg), water (0.2 kg), other ingredients (sodium chloride 2%, isolated soy protein 0.3%, and sugar 1%), and additives (sodium pyrophosphate 0.3% and L-ascorbic acid 0.05%) were mixed in a food mixer for 15 min. The ham mixtures were subjected to one of three treatments: 1) No nitrite (NN): ground hams without nitrite, 2) sodium nitrite (SN): ground hams cured with sodium nitrite, and 3) remote infusion of ANP (RANP): ground hams cured by the remote infusion of ANP for 15 min. The nitrite content in the SN and RANP treatments was controlled at 100 mg kg^{-1}. Meat batters for all three treatments were manufactured in each batch, and three independent batches were prepared on independent days. The meat batters were stored in a refrigerator at 4 °C for 12 h prior to the manufacture of the ground hams, and then filled into a plastic casing (5.0 cm in diameter) using a continuous stuffer. The ground hams were cooked in a water bath at 85 °C for 30 min until the internal temperature of the ground hams reached 75 °C. After cooking, the ground hams were cooled in ice-cold water for 1 h. Subsequently, the ground hams were stored in a refrigerator at 4 °C for 30 days. Three ground hams from each treatment/batch were randomly collected during each day of storage and used for quality property analysis.

2.2.2. Total aerobic bacteria, coliform, and Escherichia coli counts in the ground hams

The 25 g of ground ham was blended with sterile saline (225 mL) for 2 min using a stomacher (BagMixer® 400; Interscience Ind., St. Nom, France). A series of decimal dilutions were prepared using sterile saline.

For measurement of the total aerobic bacteria count, 1 mL of the initial suspension (10^{-1} dilution) was transferred to petri dishes, and 15 mL of liquid plate count agar (Difco Laboratories, Detroit, USA) was poured into each petri dish. Each dilution (0.1 mL) was spread onto the solid plate count agar (Difco Laboratories, Detroit, USA). The plates were incubated at 30 °C for 72 h, and the microbial counts were expressed as the logarithm of colony-forming units per gram (log CFU/g).

The counts of total coliform and *E. coli* were measured using *E. coli*/Coliform count plates (3 M Health Care, St. Paul, MN, USA). The 1 mL of each dilution was spread on the plate, and the plate was incubated at 37 °C for 24 h. The counts of total coliform and *E. coli* were expressed as log CFU/g.

2.2.3. Residual nitrite in the ground hams

The residual nitrite in the ground hams was measured according to AOAC method 973.31 (AOAC, 1990) with the modifications described above.

2.2.4. pH of the ground hams

The 5 g of the ground ham was homogenized with 45 mL of distilled water (T25 basic, IKA GmbH and Co. KG, Germany). The homogenates were filtered through Whatman No. 4 filter paper after centrifugation at 2090 × *g* for 15 min (Union 32R, Hanil Co., Ltd., Incheon, Korea). The pH of the filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo Inti Inc., Schwerzenbach, Switzerland).

2.2.5. Determination of the nitrosyl hemochrome content

The nitrosyl hemochrome and the total pigment contents of the ground hams were measured after extraction with 80% acetone and acidified acetone (Hornsey, 1956). For nitrosyl hemochrome determination, 10 g of ground ham was blended with 40 mL acetone and 3 mL of distilled, deionized water using a homogenizer (T25 basic). The homogenized samples were kept in the dark for 15 min before the absorbance measurement. The homogenate was filtered through Whatman No. 1 filter paper, and the absorbance of the filtrate was measured at a wavelength of 540 nm (A_{540}) using a spectrophotometer (DU®530). The nitrosyl hemochrome concentration was calculated using the following expression: Nitrosyl hemochrome concentration (ppm) = $A_{540} \times 290$. For the total pigment measurement, 10 g of the cooked samples was blended with 40 mL acetone, 1 mL HCl, and 2 mL distilled, deionized water, kept in the dark at low temperature (2–3 °C) for 1 h, and then filtered through Whatman No. 1 filter paper. The absorbance value of the filtrate was measured at a wavelength of 640 nm (A_{640}). The concentration was determined using the following expression: Total pigment (ppm) = $A_{640} \times 680$. The nitrosyl hemochrome content was expressed as the ratio of nitrosyl hemochrome to the total pigment.

2.2.6. Instrumental color measurements

The color parameters (CIE L*, a*, and b*) of each ground ham were measured using a colorimeter (CM-3500d, Minolta, Japan). Measurements were taken perpendicularly to the surface of the ground ham with an illumination area 30 mm in diameter at two different locations per sample. The results were analyzed using Spectra Magic Software (Minolta, Japan).

2.2.7. Lipid oxidation

The lipid oxidation of the ground hams was monitored by the detection of malondialdehyde (MDA). This procedure was conducted according to the method described by Jung, Nam, and Jo (2016). For this analysis, MDA was extracted from the samples with acetonitrile as follows. The ground ham sample (3 g) was homogenized with 6 mL of distilled deionized water and 50 μL of 7.2% 2,6-di-*tert*-butyl-4-methylphenol in ethanol using a homogenizer (T25 basic) at 16,000 rpm for 1 min. Next, 500 μL of the homogenate was transferred to an Eppendorf tube, and 100 μL of 6 M NaOH solution (final concentration: 1 M) was added for the alkaline hydrolysis of the protein-bound MDA. The tubes were incubated in a water bath at 60 °C for 45 min. After the tubes were cooled in ice for 5 min, 1 mL of acetonitrile was added to the tube, and the mixture was vigorously vortexed. The tube was centrifuged at 13,000 × *g* for 10 min (HM-150IV, Hanil Co., Ltd., Incheon, Korea). The upper clear phase of the supernatant contained the MDA extract. As an MDA standard, a solution of 1,1,3,3-tetraethoxypropane (3.2 mM) was diluted with deionized water to a concentration of 0.1, 0.2, 0.4, 0.8, or 1.6 μM. Subsequently, 1 mL of the MDA extract, standard, or deionized water (blank) was passed through a 0.2 μm PVDF syringe filter (Whatman), and the filtrate was collected in a vial. The concentration of MDA was analyzed via high performance liquid chromatography (HPLC, 1200 series, Agilent Tech. Inc., CA, USA), using an Atlantis T3 C18 RP column (4.6 × 250 mm, 5 μm particles) with a mobile phase consisting of 30 mM K_2HPO_4 (pH adjusted to 6.2 with H_3PO_4). The isocratic flow rate of the mobile phase was 1.2 mL min^{-1}, and the injection volume was 50 μL. The column temperature was maintained at 35 °C and the UV/Vis detector was set to a wavelength of 254 nm. The concentration of MDA in each sample was expressed in mg-MDA kg^{-1} of ground ham.

2.3. Statistical analysis

The SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. The nitrite content and temperature of the meat batter during the infusion of ANP were analyzed using the PROC REG procedure. The infusion time of ANP, operation orders, and trials were included in the regression model for the nitrite content of the meat batter. The study of the ground ham was conducted using three batches. Data from the ground ham study was analyzed using the PROC GLM procedure in a randomized complete block design (batch as a block). The experimental unit was ground ham. The statistical model included the nitrite source as the main effect. Specific comparisons were performed using 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 as $p < 0.05$.

3. Results and discussion

3.1. Properties of the meat batter remotely infused with ANP

3.1.1. Nitrite content

Plasma contains reactive nitrogen species, including nitric oxide and nitric dioxide, which are precursors of nitrite (Fridman, 2008). The formation of reactive nitrogen species in plasma is propagated by the synthesis of nitric oxide (Fridman, 2008). In ANP, nitric oxide is generated by two dominant reactions (Fig. 1). The reaction of atomic oxygen and a vibrationally excited nitrogen molecule generates nitric oxide and atomic nitrogen (R1), and the synthesis of nitric oxide via the reaction of atomic nitrogen and an oxygen molecule (R2) follows the former reaction (R1) (Fridman, 2008). These two reactions are a chain mechanism for nitric oxide synthesis in ANP. The synthesized nitric oxide is further oxidized to nitric dioxide with the reaction of ozone (*R*3) and other reactive nitrogen species (Dickenson et al., 2018; Fridman, 2008). Several studies have reported the generation of nitrite in the liquid phase after plasma treatment (Ercan et al., 2016; Jung, Lee, Lee, et al., 2017). This nitrite generation occurs via the reactions of nitric oxide or nitric dioxide with water molecules (R4–R6, Fig. 1).

In the present study, the plasma generated in the discharge source was remotely infused into the food mixer containing the meat batter. The nitrite contents of the meat batter infused with plasma were measured nine times (3 operations × 3 trials). The regression analysis showed that the order of operations had a significant effect on the nitrite generation in the meat batter ($p < 0.05$), while significant effect was not found for the trial number (Table S1, Supplementary data). The first operations of the three trials resulted in a lower generation of nitrite compared with the second and third operations (Fig. 2). The increase in the plasma discharge with the continuous supply of energy resulted in an increase in the temperatures of the electrode and plasma gas (Archambault-Caron, Gagnon, Nisol, Piyakis, & Wertheimer, 2015). The temperature of plasma gas has a quantically positive effect on the generation of nitric oxide followed by nitric dioxide, and a negative

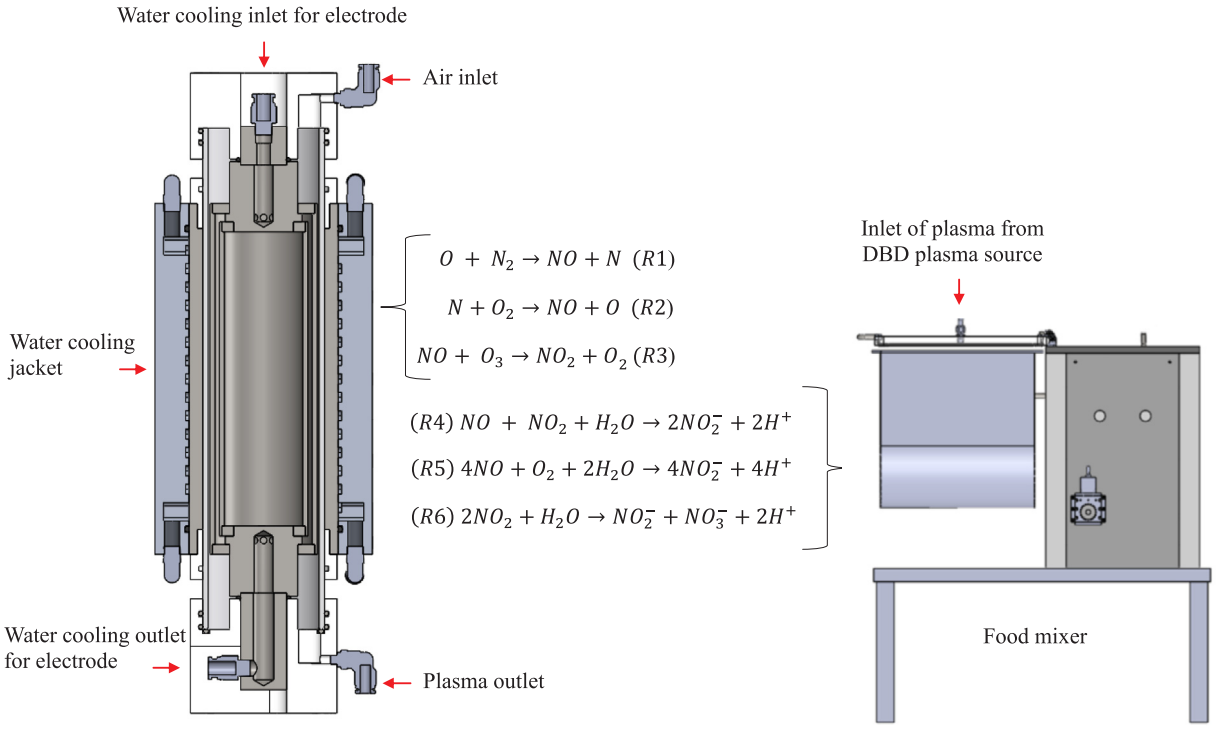

Fig. 1. System used for the remote infusion of atmospheric non-thermal plasma in this study, and the nitrite generation reactions in the meat batter.

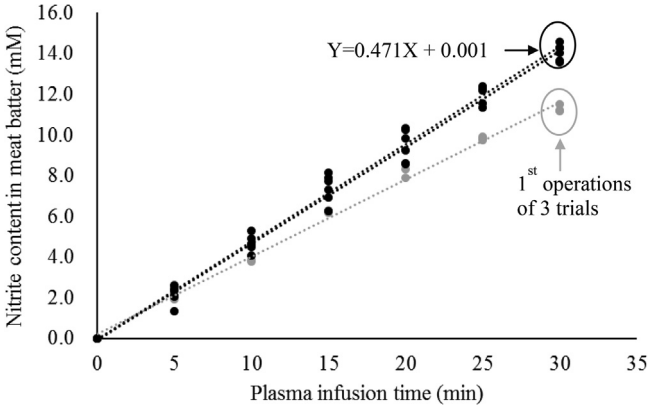

Fig. 2. Nitrite contents of the meat batter during the remote infusion of atmospheric non-thermal plasma (3 operations × 3 trials).

effect on the generation of ozone (Kim et al., 2015; Park, Choe, & Jo, 2018). The three plasma discharge operations were conducted with a 1 h interval in this study. It could be expected that the initial temperatures of the electrode in the second and third operations would be higher than in the first operation. Therefore, the plasma gas might have been warmed by reciprocal heat exchange with the electrodes, and thus produced more nitrite precursors such as nitric oxide and nitric dioxide (Archambault-Caron et al., 2015; Kim et al., 2015).

With the exception of the first operation, no significant effect of the operation order on the nitrite generation was found (Tables S2 and S3, Supplementary data). The estimated coefficient of infusion time (min) for nitrite content (mM) in the meat batter was 0.471, and the adjusted R-square value was 0.983 (Fig. 2). The time required for the generation of 100 ppm nitrite in 3.2 kg of meat batter by the remote infusion of plasma in our laboratory ANP system was 14.78 min after a pre-operation.

3.1.2. Temperature of the meat batter

The generation of plasma is accompanied by various exothermic reactions, and the continuous application of power increases the electrode temperature, which consequently increases the temperature of the plasma (Archambault-Caron et al., 2015; Dickenson et al., 2018). The ANP used in this study is also classified as cold plasma. However, the temperature of the plasma generated using ANP is generally higher than ambient temperature. Archambault-Caron et al. (2015) found a quasi-exponential rise in the temperature of the plasma from the DBD with the continuous application of power when air (~22 °C) was used as the discharge gas.

The temperature of the meat batter was measured at 5 min intervals during the mixing of the meat batter in the food mixer with or without the infusion of plasma for 20 min. The temperature of meat batter gradually increased by increase of mixing time regardless of plasma infusion (Fig. 3). The regression analysis results showed that the plasma infusion further increased the temperature of meat batter significantly (Table S4, Supplementary data). This result was similar to that of a previous study. Lee et al. (2018) found that the direct ANP treatment of 3.2 kg of meat batter resulted in a gradual increase in the meat batter

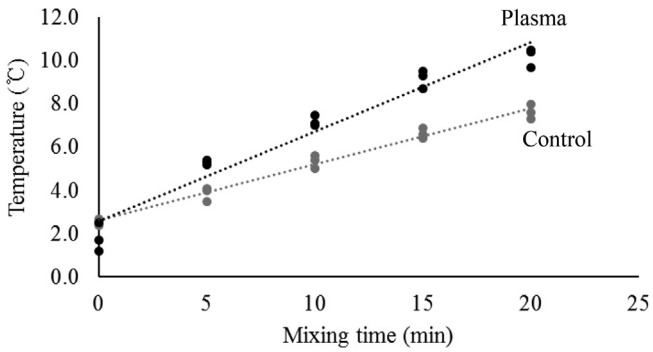

Fig. 3. Changes in the meat batter temperature with (plasma) or without (control) the remote infusion of atmospheric non-thermal plasma (3 operations × 3 trials).

temperature, and the maximum nitrite content was 42 mg kg^{-1} when the meat batter temperature was maintained at less than 13 °C after 30 min of treatment, which is the generally recommended maximum temperature for the production of meat products (Pegg & Boles, 2014). In the present study, the target level of nitrite in the meat batter was 100 mg kg^{-1}. The required plasma infusion time was 14.78 min for 3.2 kg meat batter, and the meat batter temperature was 9.2 °C after 15 min of plasma infusion. These results demonstrated that the remote infusion of plasma induced a higher rate of nitrite generation in the meat batter compared with direct plasma treatment. This result was attributed to the high power applied to the DBD source. The plasma was generated at 1.5 kW in the present study, while a power of 600 W was applied for plasma generation in the study of Lee et al. (2018). The power applied to the plasma discharge source is a critical factor in plasma generation. Increasing the applied power results in an increase in the generation of plasma, as well as in the reactive nitrogen species (Ayan et al., 2009; Dickenson et al., 2018). However, it also results in increased plasma temperature (Wang, Yang, Yao, Zhang, & Sun, 2011). Nevertheless, the meat batter temperature remained below 10 °C in this study, even though the concentration of nitrite generated in the meat batter and the applied power were higher than those in the study of Lee et al. (2018). This result could be related to the ANP treatment system. In the study of Lee et al. (2018), the DBD source was positioned on the top of the food mixer and the plasma was directly infused into the meat batter. Therefore, heat absorption from the plasma and discharge source might have increased the meat batter temperature in the previous study (Lee et al., 2018). However, in the present study, the plasma was remotely infused into the food mixer, so that the temperature of the plasma could decrease gradually during transportation. Kim et al. (2015) reported a gradual reduction of the plasma temperature with increasing distance from the end of the electrode. In addition, the DBD source was located separately from the food mixer in the present study. Therefore, the effect of plasma treatment on the temperature of the target sample was much lower using the remote plasma infusion system compared than using direct plasma treatment.

3.2. Quality properties of the ground ham cured by the remote infusion of ANP

3.2.1. Total aerobic bacteria and coliform counts

The ground hams were stored at 4 °C for 30 days. The total aerobic bacteria and coliform counts were lower than the detection limit (1 log CFU/g) regardless of the treatment used (data not shown). In the present study, the ground hams in plastic casing were stored in a refrigerator without any processing such as cutting or repackaging after cooking and cooling. Therefore, there might not be any contamination after pasteurization by heating.

3.2.2. Residual nitrite content

The residual nitrite contents of the RANP group (the ground hams cured by the remote infusion of ANP) were significantly higher than that of the SN group (ground ham cured by sodium nitrite) until 20 days of storage (Table 1; $p < 0.05$). This result was attributed to the higher nitrite content in the RANP meat batter compared to the SN meat batter. The nitrite content in the RANP meat batter was 89.87 mg kg^{-1}, while that of SN was 64.72 mg kg^{-1} before being stuffed into the casings. The addition of nitrite to the meat batter is followed by the decomposition of the nitrite into nitric oxide via several reactions (Honikel, 2008; Sebranek, 2009). However, the infusion time needed to generate 100 mg kg^{-1} nitrite in the meat batter was calculated based on the nitrite content of the meat batter at each infusion time; the amount of nitrite that decomposed into nitric oxide during the mixing of the meat batter could not be calculated. Therefore, the RANP meat batter might have contained more nitrite than the SN meat batter. The residual nitrite contents of RANP and SN gradually decreased with increasing storage time. The decrease in nitrite in cured meat products occurs via its continuous decomposition into nitric oxide, oxidation into nitrate, and combination with lipids or proteins (Honikel, 2008). A more rapid decrease in the residual nitrite was found for RANP than for SN, and there was no significant difference between the residual nitrite of SN and RANP after 30 days of storage. This result was related to the pH of the ground hams. The pH of RANP was 6.23–6.27 during storage; this value was significantly lower than those of NN and SN (Table 1). The decomposition of nitrite into nitric oxide is increased at low pH values at which nitrite is protonated, which promotes its subsequent dissociation into nitric oxide and nitric dioxide (Sebranek, 2009). The rapid decrease of nitrite in cooked meats with lower pH values has been reported previously (Honikel, 2008). The generation of nitrite via the reaction of reactive nitrogen species in the plasma with water molecules in the neutral pH region is accompanied by the release of hydrogen ions (Fig. 1R4–R6) (Jo, Lee, Lim, et al., 2018; Jung, Lee, Lee, et al., 2017; Lee et al., 2018). Therefore, the pH of the meat batter gradually decreased with increasing nitrite generation by the infusion of ANP. The RANP meat batter showed a lower pH than those of NN and SN.

Table 1
Residual nitrite content and pH of ground ham cured by the remote infusion of atmospheric pressure plasma.

Treatment[1]	Meat batter	Storage days				SEM[2]
		1	10	20	30	
		Residual nitrite content (mg kg^{-1})				
NN	0.49^{z}	0.82^{az}	0.02^{cz}	0.00^{cz}	0.12^{by}	0.019
SN	64.72^{y}	49.07^{ay}	35.09^{by}	28.20^{cy}	19.76^{dx}	0.327
RANP	89.87^{x}	61.25^{ax}	41.89^{bx}	31.17^{cx}	20.40^{dx}	0.308
SEM[3]	0.859	0.281	0.314	0.246	0.182	
		pH				
NN	6.20^{x}	6.36^{xb}	6.40^{xa}	6.38^{xb}	6.36^{yb}	0.004
SN	6.20^{x}	6.36^{xb}	6.40^{xa}	6.39^{xa}	6.40^{xa}	0.004
RANP	6.10^{y}	6.23^{yb}	6.27^{ya}	6.27^{ya}	6.27^{za}	0.003
SEM[3]	0.002	0.003	0.004	0.003	0.003	

$^{x–z}$Different letters in the same column indicate significant differences between means ($p < 0.05$).
$^{a–c}$Different letters in the same row indicate significant differences between means ($p < 0.05$).
[1] NN: ground hams without nitrite; SN: ground hams cured with sodium nitrite; RANP: ground hams cured by the remote infusion of ANP for 15 min.
[2] Standard error of the mean (n = 12).
[3] (n = 9).

The presence of nitrite in meat products has both benefits and risks. Residual nitrite content is considered a major factor in ensuring the microbiological safety of cured meat products during storage because of its antimicrobial activity (Sebranek et al., 2012). However, residual nitrite in meat products can generate a carcinogenic compound, nitrosamine, during high-temperature cooking and in the acidic conditions of the human stomach (De Mey, De Maere, Paelinck, & Fraeye, 2017). Therefore, the amount of nitrite generated in meat batter should be clearly calculated, including the amount of nitrite decomposed during the mixing of the meat batter, when ANP is used as the curing process.

3.2.3. Nitrosyl-hemochrome content

Nitrosyl-myoglobin is formed in meat batter by the combination of myoglobin and nitric oxide, which is derived from the decomposition of nitrite. Nitrosyl-myoglobin then forms nitrosyl-hemochrome when subjected to heating (Sebranek, 2009). The nitrosyl-hemochrome content of RANP was significantly higher than that of SN at 1 and 10 days of storage (Table 2; $p < 0.05$). This result may have been caused by the high nitrite content and low pH of the RANP meat batter compared with that of SN. Increased added nitrite in the meat batter and decreased meat batter pH increase the formation of nitroso-myoglobin and thus nitrosyl-hemochrome because of the increased generation of nitric

Table 2
Nitrosyl-hemochrome content and color (L*, a*, and b* values) of ground ham cured by the remote infusion of atmospheric pressure plasma.

Treatment[1]	Storage days				SEM[2]
	1	10	20	30	
	Nitrosyl-hemochrome content (%)				
NN	3.62^{z}	3.77^{z}	4.21^{y}	2.73^{y}	0.572
SN	30.04^{ay}	26.03^{by}	27.26^{abx}	25.80^{bx}	0.778
RANP	34.29^{ax}	29.38^{bx}	30.53^{bx}	24.72^{cx}	0.628
SEM[3]	0.625	0.568	0.933	0.717	
	L value*				
NN	65.43^{cxy}	70.14^{bx}	71.74^{ay}	70.94^{abx}	0.34
SN	64.45^{by}	70.21^{ax}	70.64^{axy}	69.72^{axy}	0.391
RANP	66.05^{cx}	68.45^{by}	69.75^{ay}	68.55^{by}	0.238
SEM	0.262	0.144	0.303	0.503	
	a value*				
NN	2.71^{aby}	3.00^{ay}	2.49^{by}	2.37^{by}	0.104
SN	8.05^{ax}	7.09^{bx}	6.88^{bcx}	6.38^{cx}	0.146
RANP	7.90^{ax}	7.23^{abx}	6.55^{bx}	6.59^{bx}	0.195
SEM	0.144	0.088	0.127	0.221	
	b value*				
NN	14.18^{bx}	15.66^{ax}	15.70^{ax}	15.12^{ax}	0.216
SN	9.41^{by}	13.22^{ay}	12.87^{ay}	12.80^{ay}	0.170
RANP	9.98^{by}	12.72^{az}	12.69^{ay}	12.74^{ay}	0.168
SEM	0.244	0.125	0.147	0.204	

[x–z]Different letters in the same column indicate significant differences between means ($p < 0.05$).
[a–c]Different letters in the same row indicate significant differences between means ($p < 0.05$).
[1] NN: ground hams without nitrite; SN: ground hams cured with sodium nitrite; RANP: ground hams cured by the remote infusion of ANP for 15 min.
[2] Standard error of the mean (n = 12).
[3] (n = 9).

oxide in the meat batter (Ahn & Maurer, 1989; Sebranek, 2009). However, no significant differences were found between the nitrosyl-hemochrome contents of RANP and SN after 20 days of storage due to the more rapid decrease in the nitrosyl-hemochrome content of RANP compared to that of SN. The lowest nitrosyl-hemochrome contents for both SN and RANP were observed at 30 days of storage, with nitrosyl-hemochrome levels of 85.9% and 72.1% compared to those at 1 day of storage, respectively. The apparent decrease in the nitrosyl-hemochrome content in the cured meat product during storage was caused by the decrease in absorbance at 540 nm with the structural change of nitrosyl-hemochrome (Yu, Jiao, Ma, & Sun, 2016). Yu et al. (2016) reported that the structure of nitrosyl-hemochrome was affected by the pH, and that the absorbance value of nitrosyl-hemochrome at 540 nm was not stable under weakly acidic (pH 5.7) or neutral conditions (pH 6.3). In the present study, the pH of the ground hams was lower for RANP than SN at all storage times. This lower pH might have led to the more rapid decrease in the nitrosyl-hemochrome content of RANP compared with that of SN.

3.2.4. Color

The curing of meat products via nitrite addition results in the development of a pink color characteristic of cured meat, i.e., it increases the a* value and decreases the b* value of the meat products compared to non-cured meat products (Jung et al., 2015; Sebranek, 2009). The ground hams cured by the addition of sodium nitrite and the infusion of ANP in the present study showed a cured pink color, with an increased a* value and decreased b* value compared to the NN group (Table 2). The effect of curing on the L* value of ground ham was negligible, although there were significant differences among the treatments. The a* value and b* value of SN and RANP were similar at all storage times ($P > 0.05$). Lee et al. (2018) found no significant differences in the a* and b* values of canned ground hams cured by sodium nitrite addition or ANP treatment.

Table 3
Malondialdehyde content (mg kg^{-1}) of ground ham cured by the remote infusion of atmospheric pressure plasma.

Treatment[1]	Storage days				SEM[2]
	1	10	20	30	
NN	0.73^{cx}	0.82^{bx}	0.96^{ax}	0.74^{cx}	0.017
SN	0.54^{ay}	0.54^{ay}	0.45^{by}	0.49^{aby}	0.014
RANP	0.60^{ay}	0.46^{bz}	0.47^{by}	0.49^{by}	0.024
SEM[3]	0.019	0.011	0.016	0.025	

[x–z]Different letters in the same column indicate significant differences between means ($p < 0.05$).
[a–c]Different letters in the same row indicate significant differences between means ($p < 0.05$).
[1] NN: ground hams without nitrite; SN: ground hams cured with sodium nitrite; RANP: ground hams cured by the remote infusion of ANP for 15 min.
[2] Standard error of the mean (n = 12).
[3] (n = 9).

A gradual decline in the a* values of both the RANP and SN ground hams was found during storage. Additionally, the b* values of both RANP and SN increased after 10 days of storage compared to those at 1 day of storage, while further storage did not affect their b* values. Sun and Xiong (2015) reported a gradual decrease in the a* value of cooked cured beef during nine days of storage. This discoloration of cured meat products might be related to the color changes of nitrosyl-hemochrome during storage. Yu et al. (2016) found that the color of nitrosyl-hemochrome extracted from cooked cured beef changed from red to yellow after 0.5 days, and attributed this to the instability of nitrosyl-hemochrome at conditions under the typical pH conditions of cured meat products (pH 5.5–6.5).

3.2.5. Lipid oxidation

The lipid oxidation of the ground hams was analyzed by measuring the content of MDA, which is a secondary product of lipid oxidation. The MDA contents of both RANP and SN were significantly lower than that of NN at all storage times (Table 3). This result is a general phenomenon. Many studies have reported the inhibition of lipid oxidation in meat products containing nitrite (Dutra et al., 2017; Jung et al., 2015; Lee et al., 2018). Nitrite exerts antioxidant activity via various mechanisms. Nitric oxide, which is derived from nitrite in meat products, is a lipophilic radical and can terminate the radical chain reaction of lipid oxidation by stabilizing lipid radicals such as alkyl (L·), alkoxyl (LO·), and peroxyl radicals (LOO·) (Skibsted, 2011). In addition, nitric oxide is highly reactive towards metal ions, and stabilizes myoglobin via the formation of nitrosyl-myoglobin and free iron to form nitrosyl-metal complexes in meat products (Bergamaschi & Pizza, 2011; Ford, 2004; Skibsted, 2011). Free iron is a pro-oxidant in muscle foods, especially cooked meats, in which free iron is mainly released from myoglobin due to heat denaturation and followed by the generation of hydroxyl radicals (OH·) in the presence of hydrogen peroxide via the Fenton reaction (Bergamaschi & Pizza, 2011).

No differences between the MDA contents of RANP and SN were found at any storage days except for at 10 days of storage, at which RANP had a significantly lower MDA content. A similar result was reported in a previous study, in which canned ground hams cured by ANP treatment showed similar a MDA content to those cured by the addition of sodium nitrite (Lee et al., 2018). However, plasma contains various reactive oxygen species, and plasma treatment can oxidize lipids in foods via the direct effect of free radicals and the indirect effect of the hydrogen peroxide generated in the presence of water molecules, which in turn generates hydroxyl radicals via the Fenton reaction (Afshari & Hosseini, 2014; Bergamaschi & Pizza, 2011; Ercan et al., 2016). ANP treatment has also been reported to have the opposite effect on the lipid oxidation of meat and meat products, which might have been caused by

various conditions such as different treatment times, feed gases, and types of ANP treatment (Lee et al., 2017). Kim, Yong, Park, Choe, and Jo (2013) found an increase in the lipid oxidation of pork loin after ANP treatment. More accelerated lipid oxidation was found when oxygen was used as the feed gas due to the increased reactive oxygen species in the plasma. Jayasena et al. (2015) also reported increased lipid oxidation when beef loin and pork butt in packaging were treated by ANP using nitrogen and oxygen as the feed gas. However, Bauer et al. (2017) found no increase of the lipid oxidation of beef after ANP treatment with ambient air. In addition, ANP treatment with ambient air into meat batter for 30 min showed no effect on lipid oxidation (Jung, Lee, Lim, et al., 2017). Nevertheless, the ANP was remotely infused into meat batter in the present study. Reactive oxygen species have short lifetimes, and the ozone molecules generated in the discharge region, which is the major reactive oxygen species in plasma, rapidly disappear via reaction with nitric oxide which generates reactive nitrogen species (Dickenson et al., 2018; Fridman, 2008). Therefore, the most reactive oxygen species of ANP might disappear before infusion into the meat batter during transportation in the remote ANP system.

During the storage of the ground hams, the MDA content of NN gradually increased until 20 days of storage. However, the MDA value of NN at 30 days of storage was no different than that at 1 day of storage. There was no increase in the MDA content of the SN and RANP ground hams during storage. In addition, the MDA content of RANP at 30 days of storage was significantly lower than that at 1 day of storage. MDA is the final product of lipid oxidation. However, decreases in the MDA content of meat products during storage have been reported, possibly due to a decrease in free MDA via an increase in adduct formation between MDA and other molecules such as proteins and nucleosides (Ayala, Munoz, & Arguelles, 2014; Papastergiadis, Mubiru, Van Langenhove, & De Meulenaer, 2012). Therefore, the changes in the MDA contents of the meat products might depend on the ratio of MDA production via lipid oxidation to adduct formation of via the reaction of MDA with other molecules. In the present study, the MDA could not be fully extracted from the ground hams, even though the MDA extraction from the ground hams was conducted using alkaline hydrolysis to recover protein-bound MDA. Additionally, the lipid oxidation of SN and RANP during storage might have been relatively more inhibited compared with that of NN due to the antioxidant activity of nitrite.

4. Conclusion

The remote infusion of ANP into meat batter rapidly generated nitrite with only a weak effect on the temperature of the meat batter. However, pre-operation of this system should be undertaken before use in the meat product curing process because the first operation resulted in low nitrite generation in the meat batter. The color and lipid oxidation quality characteristics of the ground hams cured by sodium nitrite and those cured using ANP were found to be similar. However, the ground ham cured by the remote infusion of ANP had a higher residual nitrite content, and its residual nitrite content decreased more rapidly during storage. Nevertheless, ANP treatment has a definite curing effect, and can be used as curing process to cure meat products without synthetic nitrites such as sodium and potassium nitrite. The remote ANP infusion system may be more applicable than direct ANP treatment in the meat product industry, because it can be easily connected to various food processing machinery and reduces side effects, such as heat damage, caused by ANP treatment.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125643.

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