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The use of atmospheric pressure plasma as a curing process for canned ground ham

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ABSTRACT

This study investigated the potential use of atmospheric pressure plasma (APP) treatment as a curing process for canned ground ham. APP treatment for 60 min while mixing increased the nitrite content in the meat batters from 0.64 to 60.50 mg kg^{-1} while the pH and the total content of aerobic bacteria in the meat batters were unchanged. The canned ground hams cured by the APP treatment for 30 min displayed no difference in their physicochemical qualities, such as nitrosyl hemochrome, color, residual nitrite, texture, lipid oxidation, and protein oxidation, compared with those of canned ground hams cured with sodium nitrite or celery powder at 42 mg kg^{-1} of nitrite. The canned ground hams cured by the APP treatment received a higher score in taste and overall acceptability than those cured with sodium nitrite. Canned ground ham can be cured by the APP treatment without nitrite additives.

1. Introduction

Cured meat products contain high levels of nitrite, which is responsible for developing cured color and flavor, reducing lipid oxidation, and inhibiting the growth of spoilage and pathogenic microorganisms, such as *Clostridium botulinum* and *Listeria monocytogenes*, in meat products (Parthasarathy & Bryan, 2012; Sebranek, 2009).

Generally, meat product industries utilize nitrite from two types of nitrite sources, namely, synthetic and natural. Synthetic additives such as sodium or potassium nitrite are easy to use and cheap. However, public concern regarding the use of synthetic food additives has increased because of reports of their toxicity (Johansson, Svartstrom, Phadnis, Engman, & Ott, 2010). Natural nitrite sources include vegetables containing nitrate, such as celery, cabbage, kale, and lettuce (Correia et al., 2010). However, natural nitrite sources have many drawbacks. The production process of natural nitrite is complex because an incubation step is necessary to convert the nitrate into nitrite in concentrated vegetable extracts using nitrate-reducing bacteria (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007). Natural nitrite sources derived from vegetables have a unique flavor and color, which can impair the sensory quality of meat products (Jung et al., 2015). In addition, Ballmer-Weber et al. (2002) reported that celery, which is widely used for natural nitrite production, could cause a food allergy. Therefore, the development of new nitrite sources is being studied.

A plasma is an ionized gas that consists of free radicals, ions, electrons, and other particles generated by electrical discharge (Afshari & Hosseini, 2014). It can be classified into high- and low-temperature plasma, also known as thermal and non-thermal plasma (Afshari & Hosseini, 2014). Among plasmas, non-thermal plasma, especially atmospheric pressure plasma (APP) has received significant attention in the food industry, as it can be used in non-thermal sterilization technology (Jayasena et al., 2015). Recently, APP has been suggested as a new curing process (Jung et al., 2017). Plasma contains various reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Ercan, Smith, Ji, Brooks, & Joshi, 2016). Nitrite species can be generated by the reaction of RNS with water molecules (Ercan et al., 2016; Oehmigen et al., 2010). Jung et al. (2015) have found that the APP treatment of water resulted in the generation of nitrite in water, and that the plasma-treated water had a similar effect to sodium nitrite when used as a nitrite source in emulsion-type sausages. In addition, the

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nitrite content in meat batters gradually increased when the meat batters were directly treated with APP (Jung et al., 2017).

Therefore, the potential use of APP as a curing process for meat products was tested in this study. The properties of meat batter, such as temperature, nitrite content, pH, and total aerobic bacterial count, were tested after the APP treatment, and the quality properties of canned ground hams manufactured with the APP treatment were also investigated.

2. Materials and methods

2.1. Properties of the meat batter after the APP treatment

2.1.1. Preparation of ground pork

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 by using a meat grinder (M-12S; Hankook Fugee Industries Co., Ltd., Hwaseong, Korea) with a 6-mm plate.

2.1.2. Direct APP treatment

The APP curing treatment for the meat batter was performed by using a plasma curing system (PCS-20N; Plasmapp Co., Ltd., Daejeon, Korea). The curing system included a mixing chamber, a plasma source chamber with a low-frequency (LF) power supply, and a gas circulating module (Fig. 1).

The mixing chamber provided the sealing space for the meat batter, and the rotating blades mingled the meat, ingredients, and additives. Two linear jet plasma sources (LJPS, Plasmapp Co., Ltd.) were installed in the plasma source chamber, and the sources were connected to the power supply in parallel. Ambient air was used as the process gas for the plasma discharge. The plasma source utilizes dielectric-barrier discharge (DBD), and it is designed to have showerheads, which provide uniform laminar flow of the process gas for a glow-like discharge (Gherardi & Massines, 2001). The cooling path formed inside the electrodes controls the process temperature and ensures a stable and reliable curing process. The activated gas is delivered into the mixing chamber by the gas circulating module, which uses a diaphragm pump and regulator to control the flow rate.

Ground pork (2.6 kg), back fat (0.4 kg), water (0.2 kg), ingredients (sodium chloride 2%, egg white powder 0.3%, and sugar 1%), and additives (sodium pyrophosphate 0.3% and L-ascorbic acid 0.05%) were mixed with the APP treatment. The plasma was discharged at a voltage of 7 kV and a frequency of 25 kHz, and the total power dissipated into the plasma was measured to be about 600 W. The flow rate of ambient air was set as $1.67 \times 10^{-4}\ m^3\ s^{-1}$ during the process. Meat batter samples were collected from three areas of the meat batter at 10-min intervals during plasma treatment over 60 min. The temperature, nitrite concentration, pH, and total aerobic bacteria count of the meat batter were measured.

2.1.3. Nitrite concentration

The collected samples of meat batters during the APP treatment were immediately stored at −70 °C until analysis. Nitrite content of the meat batters was measured according to AOAC method 973.31 (AOAC, 1990) with modifications. 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 were added. After mixing, 10 mL of 12% zinc sulfate were added to the flask and the sample mixture was 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) were added to the flask and the mixture was diluted to a volume of 200 mL with deionized water. After mixing, the solution was filtered through Whatman No. 4 filter paper (Whatman, Maidstone, UK). A 20-mL volume of the filtrate (sample solution) was transferred into 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 for complete color development. The absorbance of the solution was measured at 540 nm on a spectrophotometer (DU®530; Beckman Coulter Inc., Brea, CA), with distilled water as a reference sample. The nitrite concentration of meat batter was calculated using a standard curve prepared from absorbance readings of standard solutions of $NaNO_2$ (Sigma-Aldrich Co., St. Louis, MO).

2.1.4. pH

The pH of meat batter was immediately analyzed after sample collection. The meat batter sample (1 g) was homogenized with 9 mL of distilled water using a homogenizer (T25 basic; IKA GmbH & Co. KG, Staufen, Germany). The homogenates were filtered through Whatman

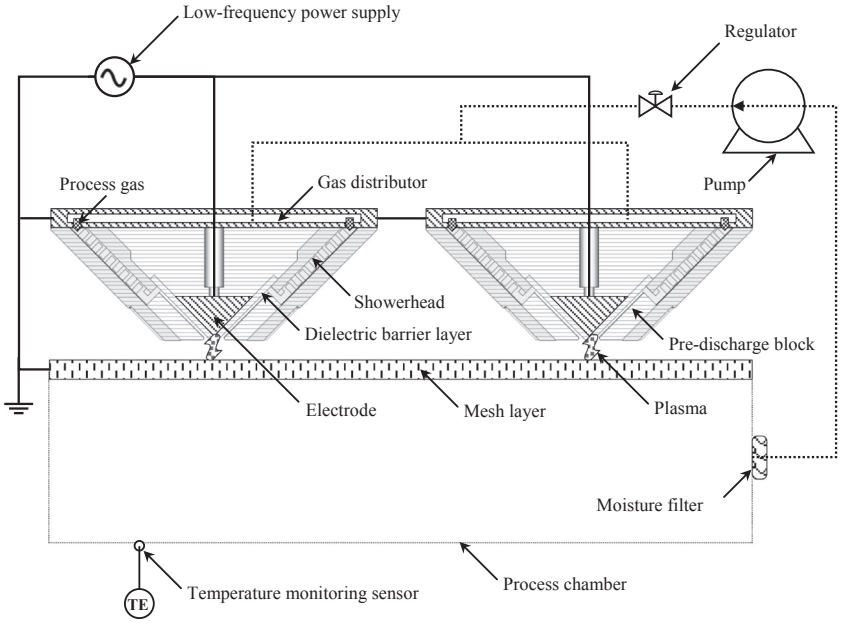

Fig. 1. Atmospheric pressure plasma system used in this study.

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 Intl Inc., Schwerzenbach, Switzerland).

2.1.5. Total aerobic bacteria

The collected samples of meat batter were immediately stored at 4 °C, and the total aerobic bacterial content in the sample was measured after storing it overnight. In a typical procedure, the meat batter sample (10 g) was blended with sterile saline solution (90 mL) for 2 min using a stomacher (BagMixer® 400; Interscience Ind., Saint-Nom-la-Bretèche, France). A series of decimal dilutions were prepared by using the sterile saline solution. The initial suspension (1 mL) was transferred into a sterile Petri dish and 15 mL of the plate count agar were poured onto the dish. Each diluent (0.1 mL) was spread on the solid plate count agar (Difco Laboratories, Detroit, MI). The plates were incubated at 37 °C for 48 h, and the microbial counts were expressed as the logarithm of colony-forming units per gram (log CFU g^{-1}).

2.2. Quality properties of caned ground ham

Based on the results from the temperature change of the meat batter after the APP treatment, a time of 30 min was decided to be optimum for the curing procedure.

2.2.1. Manufacture of canned ground hams and sample collections

Ground pork (2.6 kg) was mixed with back fat (0.4 kg), water (0.2 kg), ingredients (sodium chloride 2%, egg white powder 0.3%, and sugar 1%), and additives (sodium pyrophosphate 0.3% and L-ascorbic acid 0.05%) in a mixer for 30 min depending on the formula for each of the three treatments; 1) sodium nitrite: ground hams cured with sodium nitrite, 2) celery powder: ground hams cured with pre-converted celery powder containing 3200 ppm of nitrite, and 3) APP treatment, ground hams cured by the APP treatment for 30 min. Nitrite content in the meat batters for all treatments was controlled at 42 mg kg^{-1} because the APP treatment of meat batters for 30 min resulted in nitrite generation at a concentration of 42 mg kg^{-1} (Table 1). The meat batters for the three batches were prepared on the same day and stored in at 4 °C for 12 h prior to the manufacture of canned ground hams. The meat batter (200 g) was packed into a steel can and then sealed using an automatic closing machine (DWC-160; Duckwoo Machinery Co., Korea). Cans were placed inside the retort machine (GHPR-300; Hyupjin Co., Korea), and heated for 45 min at 118 °C ($F_0 > 5$). After the retort process, the cans were cooled in tap water for 1 h. Subsequently, the cans were dried and placed into stock. Ten canned ground hams for each treatment/batch were prepared. Three canned ground hams of each treatment/batch were randomly collected and used for analysis of the quality properties, and the remains were used for sensory analysis. After storage of canned ground hams for a day at room temperature, the instrumental color and texture of the canned ground hams were measured, and the samples for analysis of nitrosyl hemochrome, lipid oxidation, protein oxidation, and residual nitrite were collected in test tubes and stored at −70 °C until further analysis.

Table 1
Nitrite content (mg kg^{-1}), pH, and the number of total aerobic bacteria (log CFU g^{-1}) of the meat batters with atmospheric pressure plasma treatment.

Treatment time (min)	Nitrite content (mg kg^{-1})	pH	Total aerobic bacteria (log CFU g^{-1})
0	0.64[f]	6.07	2.91
10	14.73[e]	6.09	2.91
20	27.48[d]	6.06	2.96
30	42.42[c]	6.04	2.94
40	47.41[c]	6.04	2.95
50	53.84[b]	6.02	2.98
60	60.50[a]	6.03	2.98
SEM[1]	1.335	0.019	0.030

[1] Standard error of the least square mean. ($n = 18$).
[a–f] Different letters within the same column represent significant differences ($p < 0.05$).

2.2.2. Determination of nitrosyl hemochrome content

Nitrosyl hemochrome and total pigments of canned ground hams were measured, after extraction with 80% acetone and acidified acetone, respectively (Hornsey, 1956). For nitrosyl hemochrome determination, 10 g of the sample were homogenized with 40 mL of 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 measuring their absorbance. The homogenate was filtered through a Whatman No. 1 filter paper, and then the absorbance of the filtrate was measured at 540 nm using a spectrophotometer (DU®530). Nitrosyl hemochrome concentration (in ppm) was calculated as $A_{540} \times 290$. For total pigment determination, 10 g of the sample were homogenized with 40 mL of acetone, 1 mL of HCl, and 2 mL of distilled deionized water. The homogenized samples were kept in the dark at a low temperature (4 °C) for 1 h, and then filtered through a Whatman No. 1 filter paper. The absorbance of the filtrate was measured at a wavelength of 640 nm. Total pigment concentration (ppm) was calculated as $A_{640} \times 680$. Nitrosyl hemochrome content was expressed as the ratio of nitrosyl hemochrome content of the total pigment concentration.

2.2.3. Instrumental color measurements

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

2.2.4. Lipid oxidation

Lipid oxidation of canned 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 canned 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 were transferred into an Eppendorf tube, and 100 μL of 6 M NaOH solution (final concentration: 1 M) were 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 cooling 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, the 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 by HPLC (ACME 9000; Younglin Instruments Inc., Daejeon, Korea), 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 canned ground ham.

2.2.5. Protein oxidation

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with dinitrophenylhydrazine (DNPH), according to the method described by Armenteros, Morcuende, Ventanas, and Estevez (2016). Samples (1 g) were homogenized in a 1:10 (*w/v*) ratio in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using a homogenizer for 30 s. Two equal aliquots of 0.2 mL each were taken from the homogenates and dispensed in 2-mL Eppendorf tubes. Proteins were precipitated by cold 10% trichloroacetic acid (1 mL), with subsequent centrifugation for 5 min at 10,000*g* for 10 min (HM-150IV). One pellet was treated with 1 mL of 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (*w/v*) 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed twice with 1 mL ethanol:ethyl acetate (1:1, *v/v*), in order to remove excess DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer (pH 6.5) containing 6 M guanidine hydrochloric acid, stirred and centrifuged for 2 min at 10,000*g* for 10 min (HM-150IV) to remove insoluble fragments. Protein concentration was calculated from the absorption at 280 nm, using bovine serum albumin as a standard. The carbonyl content was expressed as nmol carbonyls mg^{-1} of protein, using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones.

2.2.6. Residual nitrite

Residual nitrite in canned ground hams was measured according to AOAC method 973.31 (AOAC, 1990) with modifications as described above.

2.2.7. Instrumental texture properties

Texture properties of the canned ground hams were analyzed by a two-bite system using a Texture Analyzer (Model A-XT2; Stable Micro Systems Ltd., Godalming, UK) with a compression probe (70 mm diameter) attachment. The ground ham (2 × 2 × 1.5 cm) underwent two cycles of 70% compression with a test speed of 2 mm s^{-1}. Texture characteristics of the ground hams were expressed as hardness, springiness, cohesiveness, gumminess, and chewiness.

2.2.8. Sensory evaluation

Sensory evaluation of the canned ground hams was conducted within a week of the canned ground hams being stored at room temperature and implemented using a group of ten panelists. Three sessions were conducted over three days. Each session was arranged with the canned ground hams in each batch. Canned ground hams were reheated at 180 °C for 3 min using an electric steam oven (EON-C305CSM, Tongyang Magic Co., Korea) and cut regularly. Samples of the canned ground hams were served to the panels on white glass plates. The scoring of each sample was done on a single sheet using a 9-point hedonic scale (1 = strong dislike, 9 = strong liking). The sensory parameters that were scored by the panel included color, flavor, taste, texture, and overall acceptability.

2.3. Statistical analysis

The studies of meat batter were conducted in triplicate. Three samples of the meat batter were collected and the average value of the data for the three samples was used as data for each repetition. These data were analyzed using the PROC GLM procedure that included the main effect of the treatment time of APP. The study of canned ground ham was conducted with three batches. Data from the canned ground ham study were analyzed using the PROC GLM procedure in a randomized complete block design (batch as a block). The experimental unit was canned ground ham. The statistical model included the main effect of the nitrite source. For analyzing data from the sensory evaluation, the panel was included in the model as a random effect. 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 for $p < 0.05$. SAS software (version 9.3; SAS Institute Inc., Cary, NC) was used for all statistical analyses.

3. Results and discussion

3.1. Properties of the meat batter after the APP treatment

3.1.1. Temperature of the meat batter

Non-thermal APP with DBD used in this study can be potentially applied in a variety of areas because it is easy to use and the gas temperature is lower compared with that of thermal or high-temperature plasma (Ayan et al., 2009). Although the gas temperature of non-thermal APP is not high, it can be a detrimental factor if APP is utilized for heat sensitive foods, such as muscle foods. For this reason, a cooling path was formed inside the electrodes used in this study in order to lower the gas temperature. The temperature of the meat batter was monitored at 10-min intervals during the APP treatment for 60 min. The meat batter temperature increased from approximately 1.0 °C before treatment to 2.3, 5.0, 8.9, 12.9, 17.8, and 19.4 °C (data not shown). Although the plasma system was equipped with a cooling system, the temperature behavior of the meat batter treated with APP was similar to that observed in a previous study, where Jung et al. (2017) found that the temperature of the meat batter increased from 0.2 to 20 °C during the APP treatment of 60 min without a cooling system. When a DBD plasma is generated in the air, microdischarges are formed in the gaps between the electrodes, in which all energies are dissipated, consequently increasing the gas temperature (Ayan et al., 2009). Previous studies have found that the gas temperature of the DBD plasma could be lowered by decreasing the input power (Ayan et al., 2009). However, a decrease in the input power lowered plasma production (Wang, Yang, Yao, Zhang, & Sun, 2011). The end-point temperature of a meat batter to be used for meat products is recommended to be between 10 °C and 13 °C (Pegg & Boles, 2014). Therefore, in the following experiments, the APP treatment of a meat batter for the manufacture of canned ground hams was conducted up to only 30 min.

3.1.2. Nitrite level and pH

Atmospheric oxygen and nitrogen molecules are dissociated by energetic electrons in the discharge layer, which generate RNS and ROS in the gas-phase discharges of APP (Thomas & Vanderschuren, 1999). The nitrogen oxides, such as NO, NO_2, N_2O_3, N_2O_4, and N_2O_5 that are present among the generated RNS, are known to participate in the generation of nitrite after diffusion into the sample containing water by the following reactions (Oehmigen et al., 2010; Thomas & Vanderschuren, 1999);

$$NO + NO_2 + H_2O \rightarrow 2NO_2^- + 2H^+ \quad (1)$$

$$2NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \quad (2)$$

$$N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+ \quad (3)$$

$$N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \quad (4)$$

$$4NO + O_2 + 2H_2O \rightarrow 4NO_2^- + 4H^+ \quad (5)$$

Various studies have found an increase in the nitrite concentration in the liquid after plasma treatment (Ercan et al., 2016; Jung et al., 2015; Oehmigen et al., 2010). Recently, Jung et al. (2017) reported a gradual increase of nitrite content in meat batters with increasing APP treatment time. This behavior was similar to that observed in the present study. The APP treatment of meat batters resulted in the generation of nitrite in meat batters and consequently the nitrite content of meat batters was significantly increased from 0.64 to 60.50 mg kg^{-1} after the APP treatment for 60 min (Table 1, $p < 0.05$). The meat batters

treated with APP for 30 min contained 42 mg kg^{-1} of nitrite.

A decrease in the pH of liquids is a general occurrence when nitrite is generated after the diffusion of RNS from the gas-phase discharges into the liquids, since hydrogen ions are released together with nitrite generation (Oehmigen et al., 2010; Rayson, Mackie, Kenndy, & Dlugogorshi, 2012). Jung et al. (2015) have found that the pH of water decreased from 7 to 2–3 with the APP treatment. A decrease in the pH of the meat batter after the APP treatment can be problematic when APP is used for nitrite generation in meat batters. Nitrite is unstable when it is exposed to acidic conditions, forming nitrous acid that subsequently decomposes to nitrogen oxides (Braida & Ong, 2000; Rayson et al., 2012). A previous study found that the decomposition of nitrite into nitrogen oxides occurred in liquids with a pH less than 6 (Braida & Ong, 2000). In addition, the pH of meat batters is an important factor for determining the quality of the meat products. The decrease in pH near the isoelectric points of muscle proteins such as myosin and actin results in a reduced water-holding capacity of the muscle because of the lowering of net charges among the muscle proteins (Huff-Lonergan & Lonergan, 2005). Meat products manufactured with meat having poor water-holding capacity have low yields with high cooking losses and undesirable texture properties (Lee et al., 2015). Jung et al. (2017) have found that the pH of meat batters decreased from 6.00 to 5.92 when treated with APP for 30 min, although the acidification rate was not as high as that observed in liquids (Oehmigen et al., 2010). However, in this study, no significant differences were observed in the pH of meat batters following the APP treatment for 60 min (Table 1). This result may be explained by the pH buffering capacity of the meat batter and phosphates (Jung et al., 2017; Puolanne & Kivikari, 2000).

3.1.3. Total aerobic bacteria count

The total aerobic bacteria content in the meat batter was not affected by the APP treatment for 60 min (Table 1). It is well known that reactive species in plasma may cause microbial death by disruption of the cell membrane and DNA modification of microorganisms (Moisan et al., 2001). However, the antimicrobial activity of APP is influenced by various factors, such as input power, composition of the discharge gas, exposure time, and the distance between the discharge layer and microorganisms (Afshari & Hosseini, 2014). In this study, the meat batters simultaneously underwent the APP treatment with a mixing process. The distance between the discharge layer and the meat batter was *ca.* 20 cm. Attri et al. (2015) have reported that the reactive species in plasma have life times of less than 2.7 μs. Therefore, the aerobic bacteria in meat batter may not be sufficiently exposed to the reactive species. Nitrite can also inhibit the growth of microorganisms by disrupting the metabolic enzyme activity, breaking the proton gradient, and limiting their oxygen uptake (Tompkin, 2005). Furthermore, nitric oxide derived from nitrite can limit the availability of iron, which is required for bacterial metabolism, by binding to iron (Pradhan et al., 2009). In this study, the nitrite content in the meat batter was found to have increased to 60.5 mg kg^{-1} after APP treatment for 60 min. However, there was no corresponding decrease in the total aerobic bacteria count.

3.2. Properties of the canned ground ham cured by the APP treatment

3.2.1. Nitrosyl hemochrome content and instrumental color

Canned ground hams were manufactured with meat batters containing 42 mg kg^{-1} of nitrite because of the APP treatment for 30 min, addition of sodium nitrite, or addition of celery power.

The major function of nitrite in meat products is the development of the cured pink color. This action starts with the generation of nitric oxide from nitrite (Sebranek, 2009), which may occur via two possible routes (Sebranek, 2009). When nitrite is exposed to a pH of less than 6, it forms nitrous acid, which subsequently decomposes to nitric oxide. In addition, nitrite can oxidize myoglobin (Fe^{2+}) into metmyoglobin (Fe^{3+}) while being simultaneously reduced to nitric oxide. The latter combines with the iron from myoglobin and metmyoglobin to form the nitrosomyoglobin complex, which is subsequently converted to nitrosyl hemochrome with the development of the cured pink color during thermal processing (Sebranek, 2009). The nitrosyl hemochrome contents of the canned ground hams cured with sodium nitrite, celery powder, and the APP treatment were 45.25, 46.36, and 39.01%, respectively, showing no statistically significant difference (Table 2, $p > 0.05$). In addition, no significant differences were found between the colors (L∗, a∗, and b∗ values) arising from each treatment.

Table 2
Nitrosylhemochrome (%) and color (CIE L*, a*, and b*) of canned ground hams cured with sodium nitrite, celery powder, and atmospheric pressure plasma treatment.

Treatments	Nitrosyl hemochrome (%)	L* value	a* value	b* value
Sodium nitrite	45.25	69.37	6.31	12.14
Celery powder	46.36	68.98	6.65	12.69
APP[1]	39.01	69.63	6.67	12.17
SEM[2]	5.096	0.295	0.180	0.372

[1] Atmospheric pressure plasma.
[2] Standard error of the least square mean ($n = 9$).

3.2.2. Residual nitrite content

Residual nitrite contents of all canned ground hams were ca. 5.5–7.9% of their initial concentrations, with no significant differences observed among treatments (data not shown). After addition of nitrite into the meat batter, it decomposes into nitric oxide, which can bind to myoglobin, lipids, and proteins during processing and storage. Overall, this sequence of transformations results in a decrease in the nitrite concentration (Honikel, 2008). Previous studies have reported that about 35% of the initial nitrite concentration remains in meat products after manufacture (Honikel, 2008; Jung et al., 2015). However, the loss of nitrite in the canned ground hams was higher in this study compared with that reported in previous studies. The higher loss of nitrite in the canned ground hams might be related to the lower initial nitrite concentration and the higher heating temperature compared with the previous studies. Honikel (2008) reported that the residual amount of nitrite in the meat products is directly related to the initial nitrite concentration, and inversely correlated to the heating temperature.

3.2.3. Lipid and protein oxidation

The ROS and RNS present in plasma are the key factors governing microbial death and nitrite generation (Afshari & Hosseini, 2014; Jung et al., 2017). However, ROS and in particular free radicals, initiate a radical chain reaction of lipid and protein oxidation by abstracting hydrogen ions from lipid and protein molecules (Armenteros et al., 2016; Lund, Heinonen, Baron, & Estevez, 2011). Previous studies have found that the APP treatment of liquids increases the hydrogen peroxide content (Ercan et al., 2016; Oehmigen et al., 2010). Hydrogen peroxide decomposes into hydroxyl radicals by the Fenton reaction, with free iron ions that are released from either the heme or non-heme compounds present in meat and meat products (Jung, Nam, Ahn, Kim, & Jo, 2013; Stadtman & Levine, 2003). In this study, the malondialdehyde (a secondary oxidation product of lipids) contents of the canned ground hams cured with sodium nitrite, celery powder, and the APP treatment were 0.55, 0.53, and 0.52 mg kg^{-1}, respectively (Fig. 2), indicating that there was no significant difference among these treatment methods ($p > 0.05$). However, contrary results regarding the effect of the APP treatment on the lipid oxidation of meat and meat products have been reported. Jayasena et al. (2015) found that lipid oxidation in pork and beef increases after APP treatment. Kim et al. (2011) reported an increase in lipid oxidation of bacon treated with APP after storage for 7 days compared to that in a non-treated sample. However, no increase in lipid oxidation was found when meat batters were treated with APP (Jung et al., 2017).

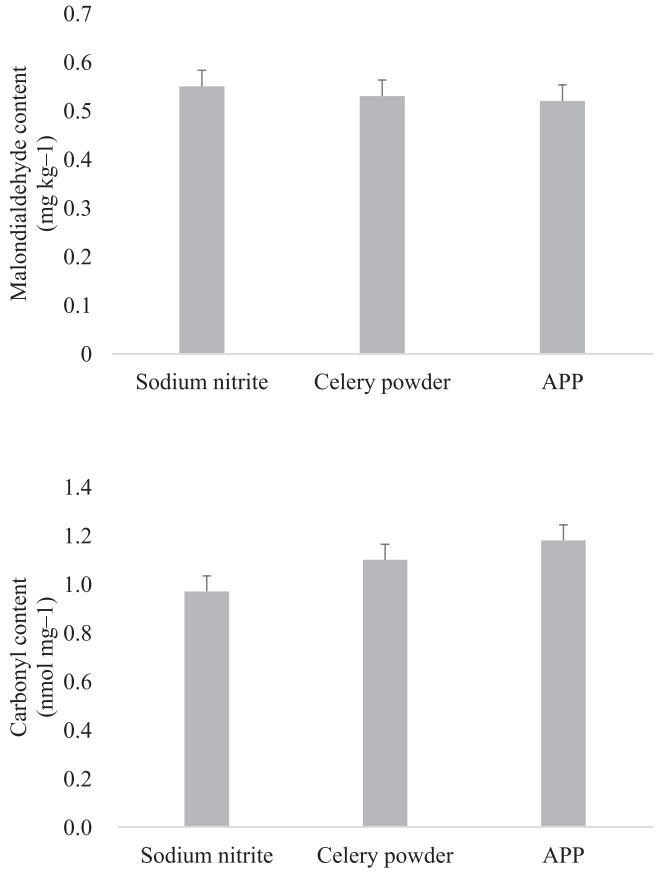

Fig. 2. Malondialdehyde (mg kg^{-1}) and carbonyl contents (nmol mg^{-1} of protein) of canned ground hams cured with sodium nitrite, celery powder, and atmospheric pressure plasma (APP) treatment.

Proteins are also susceptible to oxidation from ROS (Lund et al., 2011). Among the different proteins found in muscle foods, myosin is known to be the most sensitive to oxidation (Martinaud et al., 1997). The process of protein oxidation is similar to the radical chain reaction of lipid oxidation, albeit it is more complex and results in a larger variety of oxidation products (Lund et al., 2011). The oxidation products of proteins catalyzed by free radicals or transition metal ions, especially free iron ions, consist mainly of carbonyl derivatives, which are typically generated in the side-chains of amino acid residues such as arginine, lysine, threonine, and proline (Stadtman & Levine, 2003). Furthermore, malondialdehyde leads the generation of carbonyls by reacting with the lysine residues of proteins (Lund et al., 2011). The carbonyl content in the canned ground hams cured by the APP treatment was found to be 1.18 nmol mg^{-1} of protein, which was not significantly different to the amount measured in the canned ground hams cured with either sodium nitrite (0.97 nmol mg^{-1}) or celery powder (1.10 nmol mg^{-1}) (Fig. 2; $p > 0.05$).

These results show that the APP treatment of meat batters did not influence the oxidation levels of lipids and proteins in the canned ground hams. The oxidation of lipids and proteins in the canned ground hams is generally controlled by the nitrite and ascorbic acid present in meat batters, as both of these compounds are antioxidants. Although nitrite is an oxidizing agent, nitric oxide derived from nitrite can act as an antioxidant by binding oxygen molecules, free iron ions, and lipid radicals that are the intermediate products of the lipid oxidation process (Honikel, 2008). However, nitric oxide can also act as an oxidant in the presence of superoxide radicals, by forming peroxynitrite and then peroxynitrous acid, which can dissociate into nitrogen dioxide and hydroxyl radicals (Repetto, Semprine, & Boveris, 2012). Ascorbic acid can stabilize free radicals and lipid radicals by donating electrons (Nimse & Pal, 2015). In addition, ascorbic acid catalyzes the reduction of nitrite to nitric oxide (Sebranek, 2009). Berardo et al. (2016) found that lipid oxidation in sausages was inhibited by the addition of nitrite and ascorbic acid, while protein oxidation was unaffected.

3.2.4. Instrumental texture properties

The hardness, springiness, cohesiveness, gumminess, and chewiness of the canned ground hams did not differ among the different treatment methods (Table 3). Nitrous acid formed from nitrite in the meat batter can react with the sulfhydryl groups in proteins, to form disulfide bonds, which influence the textural properties of the meat product (Pegg & Shahidi, 2000). In this study, nitrite was added into the meat batter in equal amounts in all treatments. In addition, protein oxidation in meat products results in an increase of hardness because of the formation of cross-links between the protein molecules (Lund et al., 2011). In this study, no difference in protein oxidation was observed among the different treatments. Jayasena et al. (2015) have reported that there is no influence of the APP treatment on texture properties of pork and beef. However, there are limited data on the effect of the APP treatment on the texture of meat and meat products.

3.2.5. Sensory evaluation

The preference for visual color and odor of canned ground hams was tested by sensory panels. The instrumental color and texture of the canned ground hams were no different among the different treatments. Sensory panels gave similar scores to visual color and texture of the canned ground hams cured with sodium nitrite, celery powder, and APP (Table 4, $p > 0.05$). The meat products cured with nitrite have a distinctive cured flavor because of the various volatile nitrogen-containing compounds (Pegg & Shahidi, 2000). The canned ground hams cured by the APP treatment received a score of 5.37 for flavor, which was not significantly different to the scores of 4.16 and 5.32 received by the canned ground hams cured with sodium nitrite and celery powder, respectively ($p > 0.05$). However, there were significant differences between the scores for the taste of the canned ground hams ($p < 0.05$). The canned ground hams cured with sodium nitrite received a lower score in taste than those cured with celery powder and the APP treatment. Consequently, the score of overall acceptability was significantly lower in the canned ground hams cured with sodium nitrite than others ($p < 0.05$). A previous study reported the undesirable sensorial quality of sausages cured with 23 g kg^{-1} of celery powder compared with that of sausage cured with sodium nitrite (Jung et al., 2015). However, in this study, the canned ground hams cured with 14 g kg^{-1} of celery

Table 3
Texture profile of canned ground hams cured with sodium nitrite, celery powder, and atmospheric pressure plasma treatment.

Treatments	Hardness (N/cm^2)	Springiness	Cohesiveness	Gumminess	Chewiness
Sodium nitrite	50.28	0.60	0.21	10.56	6.39
Celery powder	47.90	0.63	0.21	10.35	6.49
APP[1]	50.10	0.68	0.22	10.90	7.35
SEM[2]	2.086	0.027	0.011	0.819	0.628

[1] Atmospheric pressure plasma.
[2] Standard error of the least square mean ($n = 9$).

Table 4
Sensory evaluation of canned ground hams cured with sodium nitrite, celery powder, and atmospheric pressure plasma treatment.

Treatments	Color	Flavor	Taste	Texture	Overall acceptability
Sodium nitrite	4.89	4.16	3.95[b]	5.14	4.31[b]
Celery powder	5.04	5.32	5.01[a]	5.35	5.31[a]
APP[1]	4.99	5.37	5.06[a]	5.25	5.26[a]
SEM[2]	0.211	0.270	0.277	0.300	0.267

[1] Atmospheric pressure plasma.
[2] Standard error of the least square mean (n = 9).
[a,b] Different letters within the same column represent significant differences ($p < 0.05$).

powder did not possess any undesirable sensorial quality. These results indicate that no adverse effects on the sensorial quality of canned ground ham were produced in the APP treatment of the meat batter.

4. Conclusion

In this study, APP was generated in ambient air without the supplementation of a discharge gas and utilized for curing meat products. It was found that nitrite was generated in the meat batter after the APP treatment. The canned ground hams manufactured from the meat batter treated with APP showed no undesirable properties in terms of lipid and protein oxidation, or sensorial quality, compared to those cured by sodium nitrite or celery powder. Therefore, it was concluded that the APP treatment can be used as a curing process for meat products, generating nitrite without adverse effects. The APP technology enables the manufacturing industry to cure meat products without nitrite additives.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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