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Direct infusion of nitrite into meat batter by atmospheric pressure plasma treatment

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ABSTRACT

This study investigated the influence of direct atmospheric pressure plasma (APP) treatment on nitrite levels and physiochemical quality of meat batter during the mixing process. A compact APP system was developed for installation on top of a food mixer. Meat batter composed of pork, water and sodium chloride (80:20:1, w/w/w) was treated with APP during mixing. Plasma treatment gradually increased the temperature of meat batter over 60 min from 0.2 °C to 20 °C. Total aerobic bacterial count of meat batter was not influenced by plasma treatment for 30 min ($p > 0.05$). The nitrite level in meat batter increased steadily with increasing plasma treatment duration ($p < 0.05$), reaching 65.96 ppm at 30 min. Consequently, the CIE a^*- and b^*-values of cooked meat batter gradually increased and decreased, respectively, as the time of plasma treatment increased. According to the results, direct APP treatment can replace nitrite addition in cured meat processing.

1. Introduction

The process of curing improves sensorial quality, shelf life, and safety of processed meats by adding ingredients such as salt, sugar, spice, nitrite, and/or nitrate (Sebranek, 2009). Nitrite is an essential curing ingredient because of its multifunctional role. Nitrite develops color and flavor, inhibits lipid oxidation, and limits the growth of spoilage and pathogenic microorganisms including *Clostridium botulinum* in processed meats (Pegg & Shahidi, 2000).

Recently, the increase of consumer demand for natural and organic food has precipitated the development of natural curing processes for processed meat (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). For natural curing processes, natural nitrite sources such as vegetable juice concentrate containing nitrate with starter cultures that reduce nitrate to nitrite, or pre-converted (nitrate to nitrite) vegetable concentrate are used instead of chemical nitrite sources such as sodium nitrite (Sebranek et al., 2012; Parthasarathy & Bryan, 2012). However, a critical problem in the use of vegetable concentrates for curing has emerged. Jackson, Sullivan, Kulchaiyawat, Sebranek, and Dickson (2011) reported that the microbial safety of naturally cured meat products was lower than that of sodium nitrite cured meat products. The concentration of added and residual nitrite in processed meat is important for limiting microorganism growth (Jackson et al., 2011; Xi, Sullivan, Jackson, Zhou, & Sebranek, 2011). Nevertheless, the amount of nitrite added to processed meat by vegetable concentrates is lower than when sodium nitrite is used, due to the natural pigments and flavors they contain that cause undesirable sensory characteristics. Therefore, the optimal concentration of vegetable concentrate added into processed meats is about 0.3 g kg^{-1} of formulation (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007; Sebranek et al., 2012; Jung et al., 2015a). In addition, vegetable concentrates typically contain nitrite at a concentration of 15,000–20,000 mg kg^{-1} (Sebranek et al., 2012). Therefore, the added concentration of nitrite into processed meat by vegetable concentrate is only about 45–60 mg kg^{-1}, which is not enough to assure microbial safety (Sebranek et al., 2012).

Plasma is ionized gas, literally defined as the fourth state of matter, along with solids, liquids, and gases (Thirumdas, Sarangapani, & Annapure, 2015). Plasma technology has been variously used in food processing for non-thermal sterilization, surface modification of packaging materials, increasing biological activity of natural compounds, and water purification (Foster, Sommers, Gucker, Blankson, & Adamovsky, 2012; Kim et al., 2014; Thirumdas et al., 2015; Jayasena et al., 2015; Kim et al., 2015; Yong et al., 2015). Recently, Jung et al. (2015a,b) suggested plasma treated water as a potential nitrite source for curing of processed meat as it contains nitrogen species such as nitrate and nitrite created by plasma-liquid interaction, without change in color or flavor. Therefore, we hypothesized that plasma treatment of meat batter could generate nitrite in meat batter by interaction of plasma with the liquid in meat batter. Furthermore, this process could

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sterilize meat batter, extending shelf life of the processed meat product and ensuring food safety.

In this study, the effect of direct atmospheric pressure plasma (APP) treatment on meat batter is investigated in terms of nitrite concentration, total aerobic bacterial count, and physicochemical quality of meat batter.

2. Materials and methods

2.1. Direct APP treatment

APP treatment was carried out using a dielectric barrier discharge (DBD) plasma system (Plasmapp PCS-20 N, Plasmapp Co., Daejeon, Korea). The system was composed of a main chamber for mixing, a plasma chamber, a plasma power supply, and a gas circulating module (Fig. 1). The main chamber stored a meat product and provided a sealed space, and plasma was discharged by the power supply in the plasma chamber, which was connected to the main chamber. The discharged gas was supplied into the main chamber to cure the meat product by the gas circulating module in which a diaphragm pump of the module received the activated gas from the main chamber and provided it into the plasma chamber. The plasma generator in the plasma chamber included 16 DBD modules spaced apart from each other, and the two plasma electrodes, which had different polarities, were coated with silver (Ag) on the opposite sides of the rectangular alumina (Al_2O_3) plate of each DBD module. The modules were supported by copper (Cu) blocks at both ends of the ceramic plate, and electrodes were connected in parallel to the plasma power supply whose input power and frequency were 550 W and 25 kHz, respectively. The power generated a strong electric field near the boundary lines of the electrodes, and the plasma was discharged from the lines on both sides of the module. Ambient gas consisting mainly of nitrogen and oxygen was excited by the discharged plasma to produce reactive nitrogen species (RNS) including nitrite (NO_2), which was supplied into the meat batter composed of pork, water and sodium chloride (80:20:1, w/w/w) in the main chamber.

2.2. Temperature

The temperature of meat batter was monitored during plasma treatment for 1 h. A cable probe (thermocouple type K) was attached to the bottom of the mixer and connected to a digital thermometer (YF-160A, Koang Yee Enterprise Co., Ltd., Taipei, Taiwan). The temperature of the meat batter was recorded at 1-min intervals.

2.3. Total aerobic bacterial counts

Samples were collected from three areas of meat batter at 5 min intervals during plasma treatment over 30 min. The collected meat batter (10 g) was blended with sterile saline (90 mL) for 2 min using a stomacher (BagMixer® 400; Interscience Ind., St. Nom, France). A series of decimal dilutions were prepared using sterile saline. Each dilution (0.1 mL) was spread in triplicate onto tryptic soy agar plates (Difco Laboratories, Detroit, USA). The plates were incubated at 37 °C for 48 h, and the microbial counts were expressed as log CFU/g.

2.4. Nitrite concentration

Samples were collected from three areas of meat batter at 5 min intervals during plasma treatment over 30 min. Nitrite concentration in meat batter was measured according to AOAC method 973.31 (AOAC, 1990) with modification. Collected meat batter (10 g) was thoroughly mixed with 150 mL of warm water (80 °C) in a 250-mL volumetric flask. Afterwards, 10 mL of 0.5 mol/L. NaOH was added. After mixing, 10 mL of 120 g/L. zinc sulfate was added into the flask and 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 100 g/L. ammonium acetate (pH adjusted to 9.1 with 100 mL/L. ammonia water) was added into 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, England). A 20-mL volume of filtrate (sample solution) was transferred into a 25-mL volumetric flask, and then 1 mL of 30 mmol/L sulfanilamide in acid solution (HCl:water, 1:1, v/v) and 1 mL of 5 mmol/L N-(1-naphthyl)ethylenediamine dihydrochloride were added. 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 Instruments Inc., CA., USA) using water as a reference sample. Nitrite concentration of meat batter was calculated using a standard curve prepared from $NaNO_2$ absorbance readings (Sigma-Aldrich Co., St. Louis, MO., USA).

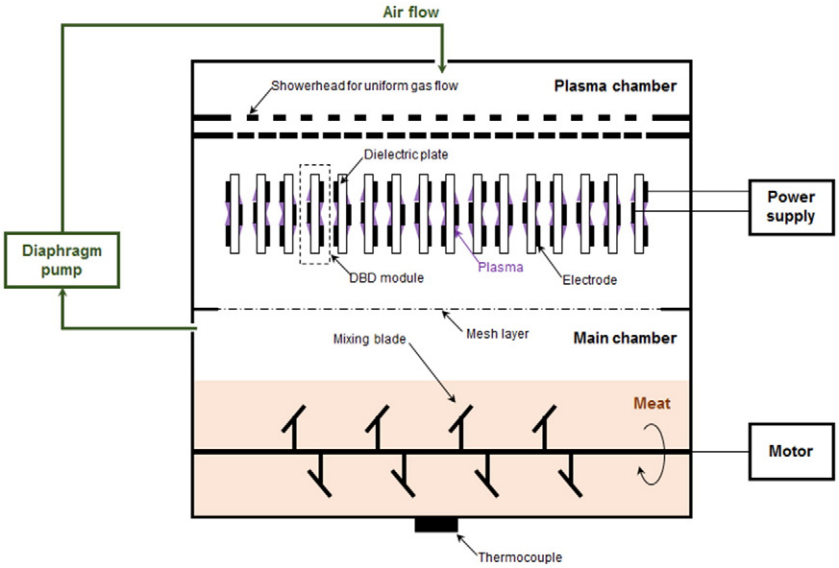

Fig. 1. Atmospheric pressure plasma system.

2.5. Color of meat batter following treatment

Collected meat batter samples of 30 g were individually vacuum-packaged (−650 mm Hg) into 20 × 15 cm low-density polyethylene/nylon vacuum bags. The packaged meat batter samples were cooked in an 85 °C water bath for 20 min and cooled in tap water for 10 min. After removal of the vacuum bag and surface moisture, the lightness (L^*), redness (a^*), and yellowness (b^*) of the cooked meat batter were measured using a spectrophotometer with the illuminant D_{65} (CM-3500d, Konica Minolta Inc., Tokyo, Japan). Measurements were taken perpendicular to the surface of cooked meat batter with an illumination area of 8 mm diameter at 2 different locations per sample.

2.6. pH and loss of mass during cooking

Meat batter samples of 1 g were homogenized with 9 mL of distilled water. 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 filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo Inti Inc., Schwerzenbach, Switzerland).

Meat batter samples of 30 g were individually vacuum-packaged (−650 mm Hg) in 20 × 15 cm low-density polyethylene/nylon vacuum bags. The packaged meat batters were cooked in an 85 °C water bath for 20 min, and cooled in tap water for 10 min. After removal of the vacuum bag and surface moisture, loss of meat batter during cooking was calculated using the weights before and after cooking.

2.7. Lipid oxidation

Lipid oxidation of meat batter was monitored by detection of malondialdehyde (MDA). This procedure was conducted according to the method described by Karatas, Karatepe, and Baysar (2002) with modifications in the MDA extraction process and in the mobile phase. For this analysis, MDA was extracted from the samples with acetonitrile as follows. A 3 g meat product sample was homogenized with 6 mL of deionized water and 50 μL of 7.2% BHT in ethanol using a homogenizer (T25b) at 16,000 rpm for 1 min. Next, 500 μL of the homogenate was transferred into an Eppendorf tube, and 100 μL of 6 mol/L. NaOH solution (final concentration 1 mol/L) was added for alkaline hydrolysis of 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 1,1,3,3-tetraethoxypropane solution (3.2 mmol/L) was diluted with deionized water to a concentration of 0.1, 0.2, 0.4, 0.8, or 1.6 μmol/L. 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 into a vial. MDA concentration was then analyzed by HPLC (ACME 9000, Younglin Instruments Inc., Daejeon, Korea). As for the analytical conditions of the HPLC, an Atlantis T3 C18 RP column (4.6 × 250 mm, 5 μm particles) was used with the mobile phase consisting of 30 mmol/L. K_2HPO_4 (pH adjusted to 6.2 with phosphoric acid). The isocratic flow rate of the mobile phase was 1.2 mL/min, 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 a sample was expressed in mg MDA/ kg meat batter.

2.8. Statistical analysis

The experiment was performed in triplicate. Data were analyzed using analysis of variance (ANOVA) in SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA). Differences among the means were assessed by Tukey's multiple comparison test. The results are reported as mean ± SD. Statistical significance was assumed at $p < 0.05$.

3. Results and discussion

3.1. Temperature

In the present study, an APP system using DBD was developed for the purpose of direct plasma curing process. Even though the plasma system was operated at low power, the APP treatment of meat batter resulted in increased temperature of the meat batter with increasing plasma treatment time (Fig. 2). The temperature of the meat batter increased from 0.2 to 20.0 °C after plasma treatment for 60 min. Generally, ice is used for the control of temperature of meat batter during the curing process. However, ice was not used in the present study, in order to investigate the effect of plasma treatment on the temperature of meat batter. The recommended end-point temperature of a meat batter that majorly consists of pork is between 10 and 13 °C (Pegg & Boles, 2014). Therefore, as the temperature of the meat batter reached 10 °C after 30 min of plasma treatment, the following experiments were conducted up to this time point.

3.2. Total aerobic bacteria count

The number of total aerobic bacteria in meat batter was monitored at 5 min intervals during plasma treatment over 30 min (Fig. 3). Plasma treatment had no influence on the number of total aerobic bacteria in meat batter ($p > 0.05$). Reactive species are key active agents in the sterilizing effect of plasma, as they cause DNA damage via oxidation (Thirumdas et al., 2015). However, the lifetime of reactive species in plasma is very short, less than 2.7 μs, and they have a very shallow

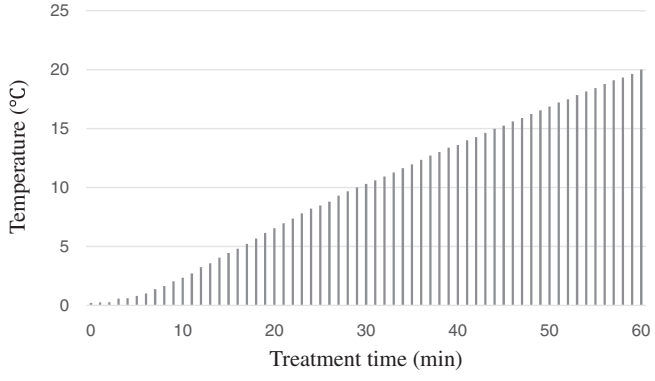

Fig. 2. Temperature (°C) of meat batter treated with atmospheric cold plasma.

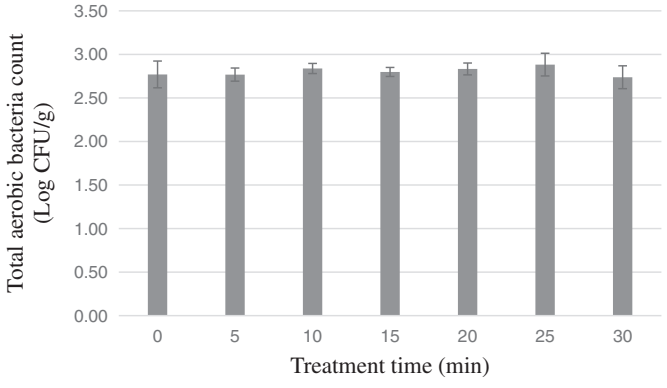

Fig. 3. Total aerobic bacteria count (log CFU/g) of meat batter treated with atmospheric cold plasma.

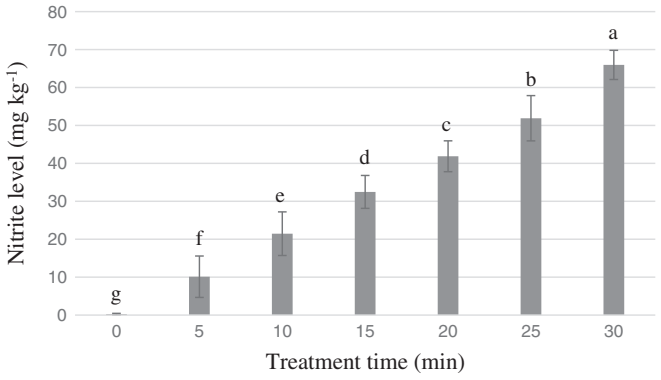

Fig. 4. Nitrite level (mg kg^{-1}) of meat batter treated with atmospheric cold plasma. [a–g]Different letters among meat batters treated with atmospheric cold plasma for different time periods (0, 5, 10, 15, 20, 25, and 30 min) differ significantly ($p < 0.05$).

penetration depth (Attri et al., 2015). Usually, the sterilizing effect of plasma is majorly affected by the exposure time of microorganisms to plasma and only occurs on the surface of a target sample (Song et al., 2009; Yong et al., 2015). In the present study, plasma treatment of meat batter was conducted simultaneously with the mixing process. Therefore, it was concluded that the exposure time of meat batter to plasma may not be sufficient to kill microorganisms.

3.3. Nitrite level

The nitrite level in meat batter gradually increased with increasing plasma treatment time ($p < 0.05$) (Fig. 4). The meat batter contained 65.96 mg kg^{-1} of nitrite after plasma treatment for 30 min. The energetic electrons in the discharge layer dissociate atmospheric nitrogen and oxygen molecules and consequently, nitrogen oxides such as NO_2, N_2O_3, and N_2O_5 form in the gas phase by several reaction pathways (Sakiyama, Graves, Chang, Shimizu, & Morfill, 2012). These nitrogen oxides react with H_2O to form nitric (HNO_3) and nitrous acids (HNO_2) which are finally decomposed into nitrate (NO_3^-) and nitrite (NO_2^-) via several chemical reactions (Thomas & Vanderschuren, 2000; Jung et al., 2015a). Oehmigen et al. (2010) reported that plasma treatment decreased pH of liquids via generation of nitric and nitrous acids. The pH of deionized water was decreased from 7 to 2– 3 after treatment with atmospheric cold plasma for 2 h (Jung et al., 2015a). Under acidic conditions, nitrite levels were decreased by the transformation of nitrite into nitrate (Oehmigen et al., 2010). However, nitrite level in plasma treated liquid was maintained in an alkaline environment by preventing transformation of nitrite into nitrate (Oehmigen et al., 2010; Jung et al., 2015a). In the present study, the pH of meat batter was slightly decreased after plasma treatment for 30 min (pH 5.92) because of the inherent buffering capacity of meat batter (Table 2). Therefore, the transformation of nitrite into nitrate could be inhibited in the meat batter. Braida and Ong (2000) reported that the transformation of nitrite into nitrate was less than 1% in solution at pH 5.8. However, the detected nitrite level in meat batter could be less than actually generated nitrite by plasma treatment because it is possible that some nitrites in the meat batter could be converted to nitric oxide during plasma treatment for 30 min.

3.4. Color of meat batter following treatment

After cooking, the cured pink color developed in all meat batter samples treated with plasma, and the negative control not treated with plasma had a brown color (data not shown). The L^* values of cooked meat batter were not significantly influenced by plasma treatment, implying that nitrite had no effect on the L^* values of cooked meat batter ($p > 0.05$, Table 1). This result is consistent with previous studies. Jung et al. (2015b) found that the L^* value of cooked meat batter cured with 70 ppm of sodium nitrite was not different to that of cooked meat batter with no nitrite added ($p > 0.05$). Horsch et al. (2014) reported that addition of 100 ppm of nitrite did not affect L^* values in processed ham ($p > 0.05$).

The a^* values of cooked meat batter were significantly increased with increasing plasma treatment time ($p < 0.05$). This result was attributed to the increase of nitrite levels with increasing plasma treatment time. In meat batter, myoglobin and nitric oxide converted from nitrite combine by several reaction pathways to form nitrosomyoglobin which produces the cured pink color (nitroso hemochrome) of processed meat after heat treatment (Pegg & Shahidi, 2000; Alahakoon, Jayasena, Ramachandra, & Jo, 2015). It is well known that redness increases, producing the characteristic cured color in processed meat after curing with nitrite. Horsch et al. (2014) found that the a^* values of ham cured with sodium nitrite and celery concentrate were higher than that of the control with no nitrite source added, and increasing nitrite concentration resulted in an increase of the a^* values of the ham.

The decrease in b^* values of cooked meat batter with increasing duration of plasma treatment was significant ($p < 0.05$). This result was proportional to the development of cured color in cooked meat batter. Horsch et al. (2014) found a decrease of b^* value in ham cured with nitrite compared to that of control with no nitrite added.

3.5. pH and loss of mass during cooking

The pH of meat batter is an important factor in determining the quality of processed meat, because the decrease in pH towards the isoelectric point of myofibrillar proteins (5.2–5.3) results in a decrease in water holding capacity and, consequently, decrease in nutritional and sensorial qualities as well as the yield of the final product due to increased loss of mass during cooking (Huff-Lonergan & Lonergan, 2005; Lee et al., 2015). The pH of meat batter was not significantly influenced until 20 min of plasma treatment ($p > 0.05$, Table 2). However, the pH of meat batter subjected to plasma treatment for 25 and 30 min was significantly lower than that of the untreated control ($p < 0.05$). Jung et al. (2015a) found that pH of deionized water was decreased from 7 to 2– 3 after treatment with atmospheric cold plasma for 2 h. The decrease of pH in plasma treated liquid is caused by the generation of nitric and nitrous acids during plasma-liquid interaction (Oehmigen et al., 2010). Although the pH of meat batter was decreased by plasma treatment, the degree of pH decline was slight (6.00 to 5.92) compared with the decrease observed in deionized water by Jung et al. (2015a). This result may be caused by the inherent buffering capacity of meat batter

Table 1
Color (L^*, a^*, and b^*) of cooked meat batter treated with atmospheric cold plasma.

	Treatment time (min)						
	0	5	10	15	20	25	30
L^*	70.29 ± 0.53	70.45 ± 0.53	70.41 ± 0.51	69.68 ± 0.95	69.98 ± 0.65	69.56 ± 0.88	69.46 ± 1.07
a^*	2.13 ± 0.57^{f}	3.98 ± 0.62^{e}	5.03 ± 0.67^{d}	5.96 ± 0.64^{c}	6.60 ± 0.54^{b}	6.98 ± 0.37^{ab}	7.20 ± 0.32^{a}
b^*	14.95 ± 0.51^{a}	13.16 ± 0.38^{b}	12.48 ± 0.66^{c}	12.31 ± 0.51^{c}	11.90 ± 0.37^{cd}	11.67 ± 0.11^{d}	11.92 ± 0.41^{cd}

Values shown are mean ± standard deviation ($n = 3$).
[a–f]Different letters within same row differ significantly ($p < 0.05$).

Table 2
pH of raw meat batter and loss of mass during cooking (%) of cooked meat batter treated with atmospheric cold plasma.

	Treatment time (min)						
	0	5	10	15	20	25	30
pH	6.00 ± 0.02[a]	5.98 ± 0.03[ab]	5.98 ± 0.03[ab]	5.97 ± 0.01[ab]	5.95 ± 0.02[ab]	5.93 ± 0.02[b]	5.92 ± 0.03[b]
Loss of mass (%)	41.52 ± 2.76	39.48 ± 3.30	38.52 ± 1.29	38.17 ± 1.89	39.84 ± 1.66	38.67 ± 1.10	39.68 ± 3.12

Values shown are mean ± standard deviation ($n = 3$).
[a,b]Different letters within same row differ significantly ($p < 0.05$).

(Puolanne & Kivikari, 2000). In addition, the loss of mass during cooking was not influenced by plasma treatment in the present study.

3.6. Lipid oxidation

Plasma contains reactive oxygen species such as hydroxyl radicals, hydrogen peroxide, and superoxide anions that play key roles in inactivation of microorganisms (Oehmigen et al., 2010; Afshari & Hosseini, 2014; Attri et al., 2015). However, these reactive oxygen species can initiate lipid oxidation (Min & Ahn, 2005). However, the plasma treatment had no effect on the MDA content of meat batters in the present study ($p > 0.05$, Fig. 5). This result is similar to that of Kim et al. (2011) who found no effect of plasma treatment on lipid oxidation in bacon. However, Jayasena et al. (2015) reported that lipid oxidation in pork butt and beef loin was increased with increasing duration of plasma treatment. This difference may be caused by the different plasma treatment system used and the short lifetime of reactive oxygen species in plasma (Attri et al., 2015).

4. Conclusion

The treatment of meat batter with APP resulted in the infusion of nitrite into meat batter and consequently the development of cured color. In addition, the lipid oxidation of meat batter was not increased by treatment of APP. However, APP had no sterilization effect in the present form. According to the results, direct APP treatment during batter mixing can be used as a replacement to nitrite addition during the curing process. If the sterilizing effect of APP is improved through further development of this system, it will have beneficial applications in the meat manufacturing process.

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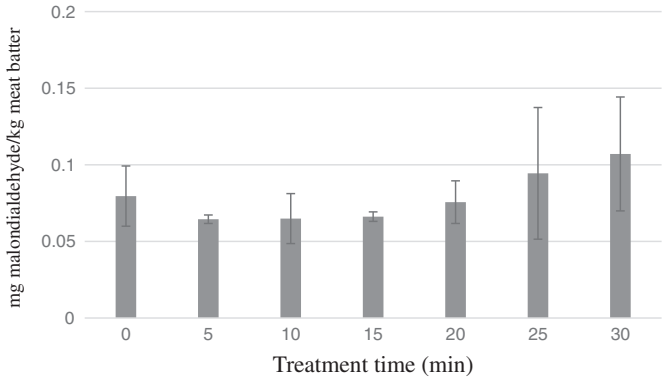

Fig. 5. Malondialdehyde content (mg kg^{-1} meat batter) in meat batter treated with atmospheric cold plasma.

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